Fiscal Year:	FY 2014	**Task Last Updated:**	FY 12/24/2013
PI Name:	Mellor, Liliana F. Ph.D.		
Project Title:	Induction of Early Stages of Osteoarthritis After Exposure to Microgravity		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Musculoskeletal Alterations Team		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Bone Fracture**:Risk of Bone Fracture due to Spaceflight-induced Changes to Bone (2) **Osteo**:Risk Of Early Onset Osteoporosis Due To Spaceflight		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	27695-7115	**Congressional District:**	4
Comments:	NOTE: formerly at Boise State University until fall 2013 (Ed., Jan 2014)		
Project Type:	GROUND	**Solicitation / Funding Source:**	2011 NSBRI-RFA-11-01 Postdoctoral Fellowships
Start Date:	11/01/2011	**End Date:**	02/28/2015
No. of Post Docs:	1	**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
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date change to 2/28/2015 per NSBRI (Ed., 12/2/14) NOTE: End date is now 11/30/2014 with PostDoc change in institution, per NSBRI (Ed., 1/15/14) NOTE: End date changed to 11/30/2013 per NSBRI (Ed., 10/24/13)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Loboa, Elizabeth (MENTOR/ North Carolina State University)		
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Performance Goal No.:			
Performance Goal Text:			

Task Description:	POSTDOCTORAL FELLOWSHIP Little is known about the effects of spaceflight on articular cartilage health, and the synovial joint in general. Unlike bone and muscle, cartilage lacks the ability to regenerate and once a catabolic cascade is triggered, it usually results in osteoarthritis. Some studies have shown degradation of the articular cartilage in response to unloading (1) and prolonged bedrest (2). However, the underlying molecular mechanisms of articular cartilage degradation in response to unloading are still elusive. My study aims to investigate the effects of simulated microgravity on chondrocytes using the RWV bioreactor. Further, in collaboration with Dr. Jeff Willey, we are studying the combined effects of radiation and simulated microgravity in articular cartilage health. My goal is to understand the molecular mechanisms involved in response to changes in gravitational forces at the cell level, and translate these changes to tissue and whole joint level. One interesting finding is that chondrocytes exposed to simulated microgravity undergo morphological rearrangement of the actin cytoskeleton. This change was not observed in irradiated cells; however, when cells were exposed to both radiation and simulated microgravity, they developed long stress-like fibers, resembling a more fibroblast morphology compared to the cortical control chondrocytes. Gene expression analyses confirmed that cells exposed to both radiation and simulated microgravity express more collagen I and less collagen II and aggrecan, which is characteristic for de-differentiated chondrocytes. The mechanotransduction mechanisms responsible for these morphological changes in response to simulated microgravity are still under investigation. Another unexpected and compelling finding is that chondrocytes respond to simulated microgravity by up-regulating sclerostin, an inhibitor of Wnt signaling known to induce bone density loss in space. However, according to a recent study, sclerostin may prevent cartilage from further degradation by decreasing production of matrix degrading genes, suggesting a chondro-protective mechanism in this tissue (3). Therapeutic treatments using an antibody against sclerostin have shown promising results to prevent bone density loss in unloading conditions; however, the effects of blocking sclerostin on neighboring tissue, such as articular cartilage, have not been addressed and may have adverse effects. In addition, irradiated chondrocytes upregulate R-spondin1, a positive regulator of Wnt signaling. R-spondin1 has been shown to protect against radiation-induced damage to the oral mucosa and intestine. Moreover, R-spondin1 has been shown to protect against inflammatory bone damage in a mouse model of arthritis (4). Our results are interesting given R-spondin1 is upregulated in response to radiation but not radiation and microgravity. If chondrocytes in normal gravity respond to radiation by producing "radioprotectant" genes, simulated microgravity is inhibiting that effect, making cartilage cells more susceptible to radiation-induced damage in microgravity conditions. This data shows that microgravity may inhibit the radio-protective mechanism of chondrocytes, suggesting a combined synergistic, degrading effect of simulated microgravity and radiation, which mimics the environment of spaceflight. Our goal for next year is to compare our results to cells incubated in hydrostatic pressure, an environment known to induce expression of anabolic genes in articular cartilage. In addition, we will study the role of primary cilia in microgravity, an important organelle involved in mechanotransduction that has also been associated with Wnt signaling pathway. 1. Niu, H.J. et al. (2012) Acta Mechanica Sinica 28, 1488-1493. 2. Liphardt, A.M. et al. (2009) Osteoarthritis Cartilage 17, 1598-1603. 3. Chan, B.Y., et al. (2011) Osteoarthritis Cartilage 19, 874-885. 4. Zhao, J. et al. (2009) Trends Biotechnol 27, 131-136.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Spaceflight has a drastic effect on the musculoskeletal system. Several studies have developed different exercise and nutrition countermeasures to prevent or minimize bone density loss and skeletal muscle atrophy after space missions. However, the effects of microgravity and radiation on articular cartilage health of the synovial joints are still elusive, and pathological conditions such as arthritis can result in severely restricted mobility. Currently, there is no cure for arthritis due to the lack of understanding of the molecular mechanisms that trigger cartilage degradation. Our study is the first to investigate the effects of radiation and simulated microgravity at the molecular level using several chondrocyte cell lines and a Rotating Wall Vessel bioreactor to simulate reduced microgravity. We found that chondrocytes respond to reduced gravity by changing their cytoskeletal morphology, and are still investigating the mechanism and mechanoreceptors responsible for re-arranging the cell morphology in response to changes in gravitational forces. Another interesting finding is the change in gene expression of molecules associated with the Wnt signaling pathway in response to simulated microgravity. The role of Wnt signaling in cartilage is well understood in embryology and development, and it has recently emerged as a critical regulator of bone and cartilage homeostasis. However, the role of Wnt signaling in arthritis is not understood, and recent studies found that sclerostin, an inhibitor of Wnt signaling, was up-regulated in mineralized cartilage and end-stage osteoarthritic samples. Sclerostin has been implicated in bone density loss in microgravity, and is now a promising therapeutic target to protect bones during space missions as well as in patients suffering from osteoporosis here on Earth. Our study is the first to detect sclerostin up-regulation in chondrocytes exposed to simulated microgravity. The mechanisms and effects of sclerostin up-regulation and changes to the Wnt signaling pathway on cartilage homeostasis are still under investigation. If Wnt signaling is in fact associated with the onset of osteoarthritis as suggested recently by a few studies, the findings from our current study may help with the understanding of the underlying mechanism involved in osteoarthritis, and can help provide a new therapeutic target for the millions of people suffering from arthritis here on Earth.
Task Progress:	Aim 1: Investigate changes in gene expression after exposure to simulated microgravity. Our data shows several changes in gene expression in response to simulated microgravity and radiation. Cells that are irradiated and exposed to simulated microgravity are de-differentiating as shown by morphological data and over-expression of collagen type I and aggrecan down-regulation. Additionally, we identified Wnt signaling as a target pathway in chondrocytes exposed to simulated microgravity. A wnt pathway PCR array evaluated 84 different genes related with Wnt pathway, and cells exposed to microgravity, expressed up-regulation of several genes that either inhibit or down-regulate Wnt signaling. In addition, qPCR analysis and ELISA confirmed up-regulation of sclerostin, a Wnt inhibitor, produced by chondrocytes in response to simulated microgravity. Aim 2: Examine changes in cell-matrix interactions in response to simulated microgravity. Preliminary data looking at CD44 expression in chondrocytes, a receptor important in cartilage homeostasis that mediates binding to hyaluronan, did not show any changes after 48 hr exposure to simulated microgravity. A time-course study will evaluate different time

	points, and other important cell surface receptors, such as integrins, will be evaluated. Aim 3: Examine the effects of simulated microgravity on the cytoskeletal morphology of chondrocytes. Chondrocytes in simulated microgravity respond by re-arranging their cytoskeletal morphology. This change is more pronounced in cells exposed to radiation and simulated microgravity. The mechanotransduction mechanisms involved with these morphological changes are still under investigation.
Bibliography Type:	Description: (Last Updated: 11/12/2020)