

<b>Fiscal Year:</b>	FY 2020	<b>Task Last Updated:</b> FY 11/24/2020	
<b>PI Name:</b>	Anderson, Morgan J Ph.D.		
<b>Project Title:</b>	Monitoring Biomarkers for Muscular Atrophy Using Nanoelectronic Chip for Astronaut Health		
<b>Division Name:</b>	Human Research		
<b>Program/Discipline:</b>			
<b>Program/Discipline--Element/Subdiscipline:</b>	TRISH--TRISH		
<b>Joint Agency Name:</b>		<b>TechPort:</b>	Yes
<b>Human Research Program Elements:</b>	None		
<b>Human Research Program Risks:</b>	None		
<b>Space Biology Element:</b>	None		
<b>Space Biology Cross-Element Discipline:</b>	None		
<b>Space Biology Special Category:</b>	None		
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<b>Zip Code:</b>	94035-0001	<b>Congressional District:</b>	18
<b>Comments:</b>			
<b>Project Type:</b>	GROUND	<b>Solicitation / Funding Source:</b>	2019 TRISH RFA-1901-PD Translational Research Institute for Space Health (TRISH) Postdoctoral Fellowships
<b>Start Date:</b>	09/01/2019	<b>End Date:</b>	08/31/2021
<b>No. of Post Docs:</b>	1	<b>No. of PhD Degrees:</b>	0
<b>No. of PhD Candidates:</b>	0	<b>No. of Master' Degrees:</b>	0
<b>No. of Master's Candidates:</b>	0	<b>No. of Bachelor's Degrees:</b>	0
<b>No. of Bachelor's Candidates:</b>	0	<b>Monitoring Center:</b>	TRISH
<b>Contact Monitor:</b>	<b>Contact Phone:</b>		
<b>Contact Email:</b>			
<b>Flight Program:</b>			
<b>Flight Assignment:</b>	NOTE: End date changed to 8/31/2021 per E. Urquieta/TRISH (Ed., 11/3/21) NOTE: End date changed to 8/31/2022 per E. Urquieta/TRISH (Ed., 7/1/21)		
<b>Key Personnel Changes/Previous PI:</b>			
<b>COI Name (Institution):</b>	Koehne, Jessica Ph.D. ( Mentor: NASA Ames Research Center )		
<b>Grant/Contract No.:</b>	NNX16AO69A-P0404		
<b>Performance Goal No.:</b>			
<b>Performance Goal Text:</b>			

**POSTDOCTORAL FELLOWSHIP**

Skeletal muscle atrophy is a serious health problem for astronauts in long-duration spaceflight under microgravity conditions. Current preventative measures and treatments against muscle atrophy require intense exercise and dietary regimens. Preemptive measurements during the onset of muscle atrophy have the potential to streamline these regimens, decreasing their daily footprint, and increasing the quality of life for astronauts. The objective of our proposed project is (1) to develop a fully integrated disposable nanoelectrode array chip (with the size of a stamp) that can be interfaced with a handheld electronic system for simultaneous detection of a panel of biomarkers to monitor the progression of skeletal muscle atrophy due to disuse under microgravity in long-duration spaceflights; and (2) to use such quantitative information to guide the combined countermeasures of physical exercise and pharmaceuticals (i.e., specific protease inhibitors) so that the intensity, duration, and frequency of exercise can be reduced.

**Task Description:**

The target biomarkers for this research are enzymatic proteases. These proteases have shown to be overexpressed for many illnesses including cancer, human immunodeficiency virus (HIV), and muscular atrophy, and operate by cleaving peptide sequences, effectively destroying critical biological proteins, such as muscle tissues. Monitoring protease biomarkers can serve as a critical early diagnostic tool for conditions specific to long term travel in microgravity. Several key factors currently limit similar healthcare diagnostics during long duration spaceflights. Instrumentation must have a small footprint, minimal power consumption, and must be simple enough for untrained users to operate accurately. Electrochemical sensors, such as the blood glucose monitor, have shown to be robust with a small instrumental footprint. To further decrease this footprint, we will use nanopatterned chips integrated to a microfluidic system to decrease the required amount of sample, minimizing the impact on user.

We will use this nanopatterned sensor to profile protease biomarkers known to be relevant to muscular atrophy and test the technique in analogs for human urine. To facilitate these measurements, we will use electrodes decorated with carbon nanofiber arrays which have been previously shown to function in complex biological media. This approach to sample collection and measurement will allow for non-invasive sample collection and will remove the need for additional chemical reagents, further decreasing the footprint of the technique. Additionally, we will use this method to demonstrate the effectiveness of protease inhibitors which may potentially serve as pharmaceutical treatments, further decreasing the need for extensive exercise regimes and dietary restrictions.

**Rationale for HRP Directed Research:****Research Impact/Earth Benefits:**

Skeletal muscle atrophy is among the most serious physiological concerns for long term space travel and habitation. The atrophy process involves a decrease in the rate of muscle cell protein synthesis accompanied by an increase the rate of protein degradation and apoptosis of various muscle components. Protease enzymes are key to the degradation of existing muscle tissue. Consequently, an early indicator of the onset of physiological muscle atrophy processes is increased activity of protease enzymes. A protease sensor would drastically improve capabilities for crew health monitoring as it would allow real time assessment into the progression of muscle atrophy and provide a framework for personalized countermeasures and treatment plans. At present, no compact point-of-care biosensors are commercially available for simultaneous detection of the enzymatic activity of multiple proteases. All of the current techniques are subject to four major limitations: 1) only one protease can be analyzed with each measurement time, 2) a large amount of sample and reagents is required, 3) a workstation in a centralized laboratory is required due to complex sample preparation, and 4) most of these techniques detect the protease concentrations rather than the enzymatic activities which may vary significantly in different biological specimens and measurement conditions. For successful integration into the Human Spaceflight Program, these limitations must be overcome. Keeping this goal in mind, we have developed a gold microelectrode array sensor for biosensing applications. We have subsequently demonstrated this sensor's applicability for multiplex detection of protease activity. In the upcoming year we will optimize the sensor for simultaneously quantify the activity of a panel of protease enzymes relevant to muscular atrophy (namely cathepsin B, cathepsin L, metalloproteinase (MMP)-2, MMP-9, calpain, and chymase) in urine. This platform can be used to establish a baseline for protease activity for an individual. Once the baseline has been established, continuous monitoring will be able to detect increases in protease activity prior to the molecular breakdown of muscle tissue. Furthermore, the resulting data can be used to guide preventative measures and treatments, thereby reducing the risks to long term space habitation. We anticipate that the application of this technology will improve astronaut health, quality of life and, importantly, crew morale.

Skeletal muscle atrophy is a serious health problem for astronauts in long-duration spaceflight under microgravity conditions. Current preventative measures and treatments against muscle atrophy require intense exercise and dietary regimens. Preemptive measurements during the onset of muscle atrophy have the potential to streamline these regimens, decreasing their daily footprint, and increasing the quality of life for astronauts. The objective of this project is to:

- (1) Develop a fully integrated disposable nano- or micro-electrode array chip (with the size of a stamp) that can be interfaced with a handheld electronic system for simultaneous detection of the a panel of biomarkers to monitor the progression of skeletal muscle atrophy due to disuse under microgravity in long-duration spaceflights;
- (2) Use such quantitative information to guide the combined countermeasures of physical exercise and pharmaceuticals (i.e., specific protease inhibitors) so that the intensity, duration and frequency of exercise can be reduced.

The target biomarkers for this research is enzymatic proteases. These proteases have shown to be overexpressed for many illnesses including cancer, HIV, and muscular atrophy, and operate by cleaving peptide sequences, effectively destroying critical biological proteins, such as muscle tissues. Monitoring protease biomarkers can serve as a critical early diagnostic tool for conditions specific to long term travel in microgravity. Several key factors currently limit similar healthcare diagnostics during long duration spaceflights. Instrumentation must have a small footprint, minimal power consumption, and must be simple enough for untrained users to operate without accurately. Electrochemical sensors, such as the blood glucose monitor, have shown to be robust with a small instrumental footprint. To further decrease this footprint, we will use nanopatterned chips integrated to a microfluidic system to decrease the required amount of sample, minimizing the impact on user. We will use this sensor to profile protease biomarkers known to be relevant to muscular atrophy and test the technique in analogs for human urine. To facilitate these measurements, we will use nano- and micro-fabricated electrodes which have been previously shown to function in complex biological media. This approach to sample collection and measurement will allow for non-invasive sample collection and will remove the need for additional chemical reagents, further decreasing the footprint of the technique. Additionally, we will use this method to demonstrate the effectiveness of protease inhibitors which may potentially serve as pharmaceutical treatments, further

**Task Progress:**

decreasing the need for extensive exercise regimes and dietary restrictions.

In the first year of this project, we have demonstrated simultaneous detection and quantification of protease biomarkers using gold microelectrode arrays with a benchtop commercial electrochemical instrument. This finding demonstrates the multiplex capabilities of this electrochemical sensor platform and shows that gold microelectrode arrays are a viable alternative to arrays of vertically aligned carbon nanofibers (VACNFs) which were originally proposed. Due to delays resulting from COVID-19, we will use these gold microelectrode arrays to expedite further development of the sensor platform and may re-examine VACNF electrode arrays in the future. Thus far, we have fabricated 400 gold microelectrode sensor chips to be used for this project and for our collaborators at Kansas State University. We have also developed a fitting model that offers improved accuracy in quantifying protease activity, as well as an algorithm for automatic, rapid data processing of all multiplex sensor data in a matter of seconds. These results have been detailed in an article published in *Biosensors and Bioelectronics* (see Bibliography section) and accompanied by an invention disclosure filed through NASA. Once in-person lab work resumes at the NASA Ames Research Center, synthesis and screening of electrochemically tagged peptide probes in artificial urine will begin for proteases candidates relevant to skeletal muscle atrophy (cathepsin B, cathepsin L, MMP-2, MMP-9, calpain, and chymase). Optimal peptide probes will be chosen based on their selectivity and their sensitivity towards the protease candidates. After optimization, the sensor chip will be evaluated in human urine samples collected at NASA Ames Research Center (ARC). The samples will be spiked with the target proteases as validation of the sensor chips and accompanying instrumentation. After validation, the sensors will be used to evaluate the efficacy of pharmaceutical treatments for muscle atrophy based on the change in their proteolytic activity.

**Bibliography Type:**

Description: (Last Updated: 04/09/2022)

**Articles in Peer-reviewed Journals**

Anderson MJ, Song Y, Fan H, Wright JG, Ren Z, Hua DH, Koehne JE, Meyyappan M, Li J. "Simultaneous, multiplex quantification of protease activities using a gold microelectrode array." *Biosens Bioelectron.* 2020 Oct 1;165:112330. Epub 2020 May 30. <https://doi.org/10.1016/j.bios.2020.112330> ; PMID: 32729476 , Oct-2020