

Fiscal Year:	FY 2020	Task Last Updated:	FY 02/07/2020
PI Name:	Mancinelli, Rocco Ph.D.		
Project Title:	BIOFILMS: Testing the Efficacy of Biofilm Formation by Antimicrobial Metal Surfaces under Spaceflight Conditions - An Effective Strategy to Prevent Microbial Biofilm Formation		
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 (2) Microbiology		
Space Biology Cross-Element Discipline:	(1) Reproductive Biology		
Space Biology Special Category:	(1) Cell Culture (2) Translational (Countermeasure) Potential (3) Bioregenerative Life Support		
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Comments:			
Project Type:	FLIGHT,GROUND	Solicitation:	2014 ILSRA--Flight Opportunities for Space Life Sciences (non-US proposers)
Start Date:	04/06/2018	End Date:	04/05/2023
No. of Post Docs:		No. of PhD Degrees:	
No. of PhD Candidates:	1	No. of Master' Degrees:	
No. of Master's Candidates:		No. of Bachelor's Degrees:	
No. of Bachelor's Candidates:		Monitoring Center:	NASA ARC
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	Rocco L. Mancinelli, Ph.D., is U.S. Co-Investigator on this German Aerospace Center (DLR), Institute of Aerospace Medicine project. Principal Investigator is Ralf Möller, Ph.D., German Aerospace Center (DLR), Institute of Aerospace Medicine, Radiation Biology Department.		
COI Name (Institution):	Möller, Ralf Ph.D. (Principal Investigator--German Aerospace Center (DLR e.V.))		
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Funding is for Dr. Rocco Mancinelli's role as U.S. Co-Investigator for this German Aerospace Center (DLR), Institute of Aerospace Medicine project, "BIOFILMS: Testing the Efficacy of Biofilm Formation by Antimicrobial Metal Surfaces under Spaceflight Conditions - An Effective Strategy to Prevent Microbial Biofilm Formation."

As Co-Investigator on the project, Dr. Mancinelli will provide his experience and expertise in microbiology and spaceflight to help design the flight experiment as well as the ground controls. He will also help trouble-shoot the system should it be necessary. He will play a major role in data interpretation, data analysis, and data management. He will help guide the ground control design and construction both on site (at the DLR) as well as remotely at NASA Ames. In addition, Mancinelli will take the lead in developing a conceptual model describing the effects of micro-gravity on the growth and development of biofilms as well as for the biofilms grown on metallic inhibitor surfaces.

To achieve many of the goals of NASA's and European Space Agency (ESA)'s space programs requires an enduring human presence in space. Long term human missions require sustained crew health and safety. A research area that is important in sustaining crew health is the development of improved spaceflight-suitable methods for microbiological monitoring, as well as contamination control and reduction. The International Space Station (ISS) is a confined and isolated habitat in an extreme, hostile environment. The human and habitat microflora varies in response to changes in environmental conditions aboard the ISS. Changes in the microflora may result in an increased health risk for the crew. Microorganisms including microbial biofilms have been found on various habitat surfaces, inside the air and water handling systems as well as the hardware used on the ISS. Biofilms are known to cause damage to equipment from polymer deterioration, metal corrosion, and bio-fouling. The primary concern regarding crew health is characterized by activity of opportunistic pathogenic microorganisms that have been noted to accumulate in the closed environments of the ISS and other spacecraft on long-duration missions. Understanding the effects of the space environment, especially altered gravity, on microbial biofilms is crucial for the success of long-term human space missions. Surface-associated biofilm communities were abundant on the Mir space station and continue to be a challenge on the ISS. The health and safety hazards linked to the development of biofilms are of particular concern due to the suppression of human immune function observed during spaceflight. Various studies have shown that certain metals reduce the number of contact-mediated microbial infections. Antimicrobial surfaces are defined as materials that contain an antimicrobial agent (such as silver, copper, and their alloys) that inhibits or reduces the ability of microorganisms to grow on the surface of a material. Antimicrobial surfaces are functionalized in a variety of different processes. The introduction of antimicrobial surfaces for medical, pharmaceutical, and industrial purposes has shown their unique potential for reducing and preventing microbial contamination. The contact killing of several types of microorganisms by copper has been assessed in multiple laboratory in-vitro studies. For sustained crew health and safety additional studies on the mechanisms involved in the formation of microbial biofilms and their efficient destruction under spaceflight conditions, i.e., long-term growth and adaptation to low gravity environments, are needed.

Task Description:

The hypothesis to be tested by this project is that surfaces containing copper and/or silver will inhibit biofilm formation under altered gravity regimes to a lesser extent than in 1 x g due to the fact that the interaction with the metal ions on the surface is slower because their movement around the cell is restricted to diffusion. The objective is to determine the effect and the rate, if any, of copper and/or silver surfaces on microbial growth rate, total biomass accumulation, and biofilm formation. The goal is to develop a conceptual model describing the effect of micro-gravity on biofilm formation grown on non-inhibiting surfaces as well as on metal surfaces that are potential biofilm growth inhibitors.

The approach will be to test three different microbial model systems (i.e., *Escherichia coli* K12, a *Staphylococcus* sp. isolate from the ISS, and the heavy metal resistant strain *Cupriavidus metallidurans* CH34) for biofilm formation on various copper- and silver-surfaces, as well as inert surfaces as controls. These surfaces differ in their antimicrobial activity based on chemical composition and/or geometric nanostructures. These surfaces will be tested for biofilm formation rates under different spaceflight relevant gravitational regimes (e.g., Moon 0.16 x g, Mars 0.38 x g, μ g ISS and 1 x g control). Microbial growth will occur under optimal biofilm-inducing conditions conducted in the KUBIK incubator inside the European Drawer Rack under defined gravitational influences. Biofilm/metal surface samples and controls will be subjected to an intense analysis program, including various microbiological, genetic, molecular biological, chemical, material-science, and structural investigations. The data generated will be of immense importance for understanding the influence of μ g and the ISS environment on biofilm formation as well as for the evaluation and production of improved antimicrobial additives, coating, components, surfaces and textiles for short- and long-term utilization for present and future astronaut/robotic-associated activities in space exploration.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Microbial biofilms are known to cause persistent infections as well as degrade a variety of materials including metals. Biofilms are notorious for their persistence and resistance to eradication. The use of antimicrobial surfaces provides an alternative strategy for inhibiting microbial growth and biofilm formation to conventional cleaning procedures and the use of disinfectants. Antimicrobial surfaces contain organic or inorganic compounds, such as antimicrobial peptides or copper and silver, that inhibit microbial growth. The objectives of this project include determining the efficacy of biofilm inhibition by different oxidation states of metals and inhibition by nanoscale texture patterns on various metals. The results from the nano-scale texture patterns represent a new technology that is applicable to inhibiting biofilm formation in hospitals, and also in the pharmaceutical and industries where biofilm corrosion is a problem.

Because of its fast generation time we used *Vibrio natriegens* as a model test organism for a variety of space environment related studies where generation time is a critical factor. Specifically, *V. natriegens* was used as a tool to study growth characteristics by determining the viable cell number and antibiotic susceptibility under simulated microgravity using a 2D clinostat (60 rpm) to establish a test system that resolves changes in microbial growth on a solid surface (agar) under microgravity. The data show that *V. natriegens* biomass increases significantly after 24 h at 37°C under simulated microgravity. The final cell population after cultivation under simulated microgravity was 60-fold greater than when cultivated under normal terrestrial gravity (1 x g). No change in susceptibility to the antibiotic rifampicin after cultivation under simulated microgravity or normal gravity was detected. These data show that *V. natriegens* is a new and innovative model organism for microbial microgravity research.

Preparative work for the upcoming EST & science verification test (SVT) tests was conducted. These tests included performing laboratory microbiology & analytical testing for sample preparation, cultivation, fixation, and potential post-flight analyses.

Task Progress:

	<p>Desiccation testing was one important part of the microbiological testing. To complete this test, samples of the ISS isolate <i>Staphylococcus capitis</i> subsp. <i>capitis</i> K1-2-2-23 and the wild type (DSM 20326) were grown to mid-log phase and washed by centrifugation. The pellets were resuspended in buffered saline to a pre-determined cell concentration, removed from the centrifugation, and aliquots dried in Eppendorf tubes. Periodically, samples of the dried organisms were tested for viability with respect to desiccation tolerance). These data from these tests illustrated that the ISS isolate is more tolerant to desiccation than the wild type.</p> <p>Additional tests were conducted to determine the viability of <i>Staphylococcus capitis</i> subsp. <i>capitis</i> K1-2-2-23 compared to the wild type (DSM 20326) when exposed to increasing levels of hydrogen peroxide. Hydrogen peroxide is an oxidizing agent that creates radical oxygen species in the cell that can kill cells and interfere with biofilm formation. The results showed that the ISS isolate is more tolerant to this oxidizing agent.</p>
Bibliography Type:	Description: (Last Updated: 02/21/2020)
Abstracts for Journals and Proceedings	<p>Siems K, Mancinelli RL, Moeller R, et al. "Staphylococcus capitis ISS isolate as a model organism for evaluating antimicrobial surfaces within the upcoming space flight experiment BIOFILMS." 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. Program and Abstracts. 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. , Nov-2019</p>
Abstracts for Journals and Proceedings	<p>Mancinelli RL, Cortesao M, Moeller R. "Microbes in Space: An overview." International Symposium on Fungi/Microbes Under Stress, San Jose de Los Campos, Brazil, May 18-26, 2019. Symposium Abstract Book, International Symposium on Fungi/Microbes Under Stress, San Jose de Los Campos, Brazil, May 18-26, 2019. , May-2019</p>
Articles in Peer-reviewed Journals	<p>Garschagen LS, Mancinelli RL, Moeller R. "Introducing <i>Vibrio natriegens</i> as a microbial model organism for microgravity research." <i>Astrobiology</i>. 2019 Oct;19(10):1211-20. https://; PubMed PMID: 31486680 , Oct-2019</p>