Fiscal Year:	FY 2023	**Task Last Updated:**	FY 02/13/2023
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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Zip Code:	94035	**Congressional District:**	18
Comments:			
Project Type:	FLIGHT,GROUND	**Solicitation / Funding Source:**	2014 ILSRA--Flight Opportunities for Space Life Sciences (non-US proposers)
Start Date:	04/06/2018	**End Date:**	04/05/2024
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:	NOTE: End date changed to 04/05/2024 per NSSC information (Ed., 2/22/23)		
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.))		
Grant/Contract No.:	80NSSC18K0751		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	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. 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, µg 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 µg 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.
	For the BIOFILMS experiment, copper and brass (CuZn37) were selected as antimicrobial surfaces, while stainless steel is used as the inert reference. All three surfaces are tested with and without tailored surface functionalization (that is, patterned grooves laser cut into the metal surfaces at 3 micron and 800 nm levels). Using the KUBIK facility in the Columbus laboratory on board the ISS, the antimicrobial efficacy of the surfaces is being tested under three different gravity levels (µg, 0.4 x g, 1 x g). [Ed. Note: KUBIK is a small incubator developed by the European Space Agency (ESA) for self-contained microgravity experiments on board the International Space Station (ISS).] Staphylococcus capitis, Cupriavidus metallidurans, and Acinetobacter radioresistens were selected as bacterial model organisms. The bacteria are incubated in specialized hardware that allows biofilm formation to occur under controlled conditions while being exposed to the different metal surfaces. The BIOFILMS experiment is being conducted during three flights on board the ISS to accommodate the complete set of sample configurations (metal type, surface functionalization, bacteria, and gravity level). The first launch of BIOFILMS was in August 2021 with SpX 23, the second in July 2022 with SpX 25, and the last flight is planned for March 2023 with SpX 27. The following are preliminary results from the first flight and part of the second, with the caveat that a complete interpretation and evaluation of the data can only be made after

Task Progress:	the completion of all three flights and post flight evaluations. The results of BIOFILMS will help to make space travel safer and more sustainable by providing new insights and design capabilities in contamination control. The Biofilms 1 data analysis is complete. It was found that patterning at both the 3 micron and 800 nm levels seems to stress the organisms, as evidenced by the production of more extracellular material (presumably mostly extracellular polysaccharides) on all metal surfaces tested. Copper shows clear inhibition of microbial growth on all types of surfaces. The brass coupon appears to inhibit microbial growth but somewhat less than copper (quantification requires further analyses). The effect of gravity is not readily apparent and appears to be masked by the effects of the metals and functionalization. It is clear that more detailed analyses need to be done. The extracellular material produced on the copper and brass plates has a more spotty/blotchy appearance than the extracellular polymeric substances (EPS) on the stainless steel plates. This might be due to the interaction of copper with the EPS. It was decided that Energy Dispersive X-Ray Analysis (EDAX) would be a good way to discern the differences and similarities. Biofilms 2 launched on SpX-25 at the end of July and we are currently working on those samples. Preliminary results appear to be similar to those from Biofilms 1. Biofilms 3 is scheduled to launch in February/March 2023.
Bibliography Type:	Description: (Last Updated: 02/22/2023)
Abstracts for Journals and Proceedings	Siems K, Müller D, Ahmed A, Van Houdt R, Mancinelli RL, Brix K, Kautenburger R, Krause J, Vukich M, Tortor A, Roesch C, Holland G, Laue M, Mücklich F, Moeller R. "Update on the spaceflight experiment “BIOFILMS”: Testing laser-structured antimicrobial surfaces under space conditions." 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. Abstracts. 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. , Nov-2022