Active Project (2013 - 2014)

Rapid Electrochemical Detection And Identification Of Microbiological And Chemical Contaminants For Manned Spaceflight Project

Center Innovation Fund: JSC CIF (Also Includes JSC IRAD) Program

Space Technology Mission Directorate (STMD)

National Aeronautics and Space Administration

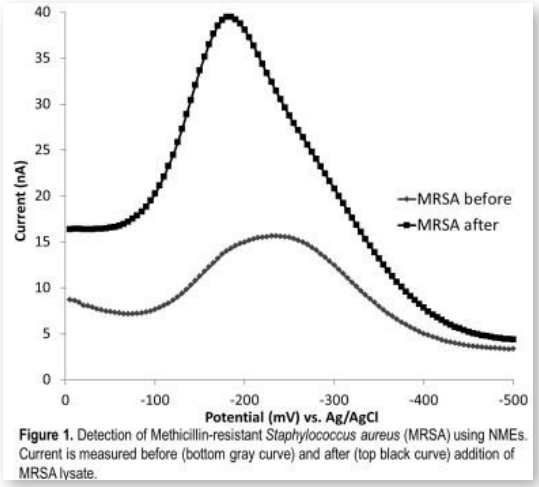
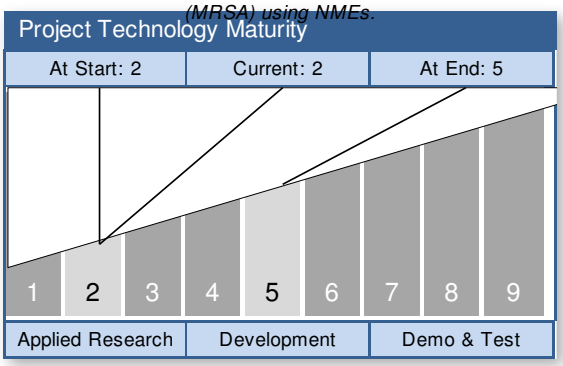


Figure 1. Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) using NMEs. Current is measured before (bottom gray curve) and after (top black curve) addition of MRSA lysate.

Detection of Methicillin-resistant Staphylococcus aureus (MRSA) using NMEs.



ABSTRACT

Microbial control in the spacecraft environment is a daunting task, especially in the presence of human crew members. Currently, assessing the potential crew health risk associated with a microbial contamination event requires return of representative environmental samples that are analyzed in a ground-based laboratory. It is therefore not currently possible to quickly identify microbes during spaceflight. This project addresses the unmet need for spaceflight-compatible microbial identification technology. The electrochemical detection and identification ...***Read more on the last page.***

Technology Area: Human Health, Life Support & Habitation Systems TA06 (Primary)
Human Exploration Destination Systems TA07 (Secondary)

ANTICIPATED BENEFITS

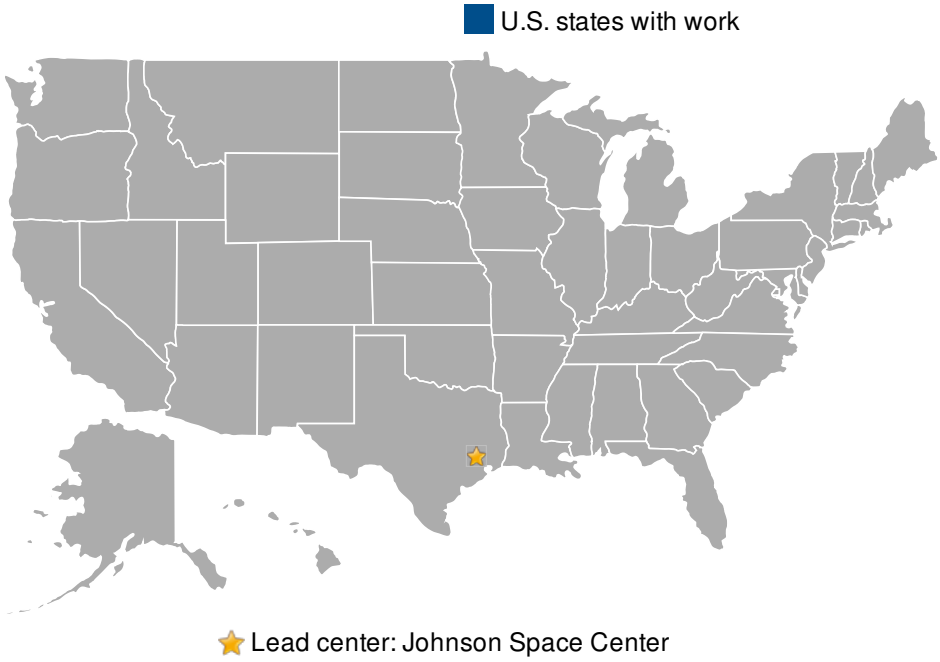
To NASA funded missions:

Currently, assessing the potential crew health risk associated with a microbial contamination event requires return of representative environmental samples.

By providing enhanced in-flight monitoring capabilities we can reduce or eliminate reliance on archival samples and improve our response to in-flight contamination events.

...

Read more on the last page.



DETAILED DESCRIPTION

A great deal of effort has gone into the development of point-of-use methods to meet the challenge of rapid bacterial identification for both environmental monitoring and clinical applications. Unfortunately, most of the methods developed rely on PCR and face inherent limitations because of the requirement for enzymatic components and thermal control. Other methods based on surface plasmon resonance, quartz crystal microbalance, and fluorescence have been reported with good detection limits, but, these methods are immunological and cannot provide genetic-level information. Further, they require labeled markers, complicated fluid handling systems, and sensitive optics that drive up cost and complexity and preclude them from outside the laboratory. Recent work by a group at the University of Toronto (U of T) has focused on developing an electrochemical platform that combines ultrasensitive detection, straightforward sample processing, and inexpensive components to create a cost-effective, user-friendly device for detection and identification of microorganisms. The platform combines an electrical cell lysis chamber, and electrochemical reporter system, and nanostructured microelectrodes (NMEs) to detect specific nucleic acid sequences. The nucleic acid sequences are unique to a given type of microorganism and can be used to identify the microorganisms ...

MANAGEMENT

Program Executive:
John Falker

Program Manager:
Ronald G Clayton

Project Manager:
James Mccoy

Principal Investigator:
Duane Pierson

Co-Investigators:
Douglas Botkin
Daniel Gazda

DETAILED DESCRIPTION (CONT'D)

present in a sample.

From the perspective of the anticipated prototype device (Lam, et al. 2012. *Polymerase Chain Reaction-Free, Sample-to-Answer Bacterial Detection in 30 Minutes with Integrated Cell Lysis*. Anal. Chem. **84(1)**: 21-5), detection of microbial contaminants will begin with a lysis chamber designed to release DNA and RNA from microorganisms present in the sample using ultrasonic or electrochemical technology. The DNA and RNA mixture is then passed into an analysis chamber containing an array of nanostructured microelectrodes (NMEs). The surface of the NMEs will be functionalized with probe molecules for DNA or RNA sequences specific to the bacteria being targeted. Binding of the DNA or RNA to the appropriate detection probe on the NME surface in the presence of an electrochemical reporter system will change the electrochemical properties of the NMEs. A potentiostat is used to measure the current at each individual electrode before and after addition of the DNA and RNA mixture. The difference in current before and after addition of the mixture to the NMEs is compared against a pre-determined threshold to check for the presence of target bacteria in the sample. The process for detection of chemical contaminants is very similar. The lysis chamber would be bypassed and the sample would flow directly into the analysis chamber. The NMEs will be functionalized with molecules to selectively bind the desired targets (analytes) and the change in the electrochemical response of each NME can again be used to detect and quantify the contaminants. Depending on the analyte of interest, it may be possible to directly measure analyte binding on the surface of the NMEs without the use of an electrochemical reporter system.

The overall project will focus on optimization of the individual aspects of the detection platform in preparation for construction of a prototype for a flight experiment. The scope of the work in this proposal is limited to characterization and optimization of the lysis step/sample preparation, probe selection, and NME structure. Lysis conditions will be optimized by evaluating parameters associated with the oscillation frequency and lysis time for ultrasonic techniques and applied voltage for the electrochemical techniques. Cell viability, as determined by fluorescent detection of DNA or RNA in live cells or by bacterial culture techniques, will be used to assess the efficiency of the methods used to release DNA and RNA from the bacterial cells. Candidate detection probes will be tested to optimize specificity of the system. Initial experiments will utilize conventional electrodes and a benchtop potentiostat to optimize analysis conditions. Functionalized electrode surfaces will also be examined using electron microscopy in an attempt to understand the role of electrode surface structure in the analysis platform. Ultrastructural characteristics such as surface morphology, structure size, and probe density will also be considered.

Initial microbial validations will be performed using microbes found during routine microbial environmental monitoring of the International Space ...

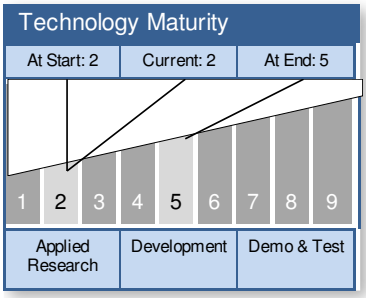
DETAILED DESCRIPTION (CONT'D)

Station (ISS) as well as related, potentially medically significant organisms. Evaluation of each group of organisms will permit optimization of target-probe interactions, and will lead to maximizing the sensitivity and specificity of the platform. Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *S. aureus* (MSSA), and the related, nonpathogenic species *S. epidermidis* will be tested to demonstrate detection of potential pathogens (MRSA and MSSA) and specificity of pathogen/non-pathogen detection (MRSA or MSSA versus *S. epidermidis*). A panel of common ISS water-borne microorganisms and related species will also be evaluated. *Cupriavidus metallidurans*, *Cupriavidus basilensis*, *Burkholderia cepacia*, *Burkholderia multivorans*, and *Ralstonia pickettii* will be utilized to demonstrate specificity for related species (*Cupriavidus* or *Burkholderia* species), detection of potential pathogens (*Burkholderia* species), and detection of potential biofouling agents (all water-borne species listed). Experiments with chemical contaminants will focus on compounds that may be present in the water produced onboard the ISS. Compounds containing functional groups that can be used for selective binding or retention on the surface of an electrode will be initially tested since they are most amenable to detection and quantification using an electrochemical platform.

After testing with a benchtop potentiostat to demonstrate the detection capability of the platform, a low-cost potentiostat will be built from commercial off-the-shelf (COTS) components and used with the optimized electrodes. Low-cost, fully functional potentiostats have emerged as a viable alternative to commercial units (Rowe, et al. 2011. *CheapStat: an open-source, "do-it-yourself" potentiostat for analytical and educational applications*. PLoS One. **6(9)**:e23783), and their relatively small production cost, footprint, mass, and power requirements suggest that they are suitable for use aboard spacecraft. The breadboard potentiostat will be paired with low-cost computing hardware, such as a Raspberry Pi or an Arduino board, and system control software such as LabVIEW, eliminating the need for ISS computing resources to operate the device. Inclusion of an automated detection step in the anticipated device is a time-saving measure to free up crew time otherwise spent visually inspecting results and evaluating the outcome of a test. The final step in the process is downsizing the optimized electrode surfaces and building NME arrays that can be controlled using the potentiostat system built in-house.

TECHNOLOGY DETAILS

An Electrochemical Approach to In-situ, Real-time Detection and Identification of Microorganisms for Manned Spaceflight



TECHNOLOGY DESCRIPTION

Recent work by a group at the University of Toronto (U of T) has focused on developing an electrochemical platform that combines ultrasensitive detection, straightforward sample processing, and inexpensive components to create a cost-effective, user-friendly device for detection and identification of microorganisms. The platform combines an electrical cell lysis chamber, and electrochemical reporter system, and nanostructured microelectrodes (NMEs) to detect specific nucleic acid sequences. The nucleic acid sequences are unique to a given type of microorganism and can be used to identify the microorganisms present in a sample.

This technology is categorized as a hardware system for manned spaceflight

- Technology Area
  - TA06 Human Health, Life Support & Habitation Systems (Primary)
  - TA07 Human Exploration Destination Systems (Secondary)

CAPABILITIES PROVIDED

This analytical platform has the potential to significantly enhance both environmental and medical monitoring capabilities for manned spaceflight. It is a non-traditional approach to detection and identification of microorganisms that overcomes many of the limitations of standard techniques such as the need for time-consuming culture steps and the use of harmful chemicals. The intended product of this work is a detailed assessment of the electrochemical approach to the genetic identification of bacteria. This approach is radically different from the often employed polymerase chain reaction method. The technology is expected to provide a sensitive, specific, and rapid sample-to-answer capability for in-flight microbial monitoring that can distinguish between related microorganisms (pathogens and non-pathogens) and chemical contaminants. Further, the project is expected to eliminate the need for sample return while significantly reducing crew time required for detection of multiple targets

...

Performance Metrics		
Metric	Unit	Quantity
sample-to-answer capability	minutes	30

TECHNOLOGY DETAILS

POTENTIAL APPLICATIONS (CONT'D)

The low cost, low-power, deployable, point of use microbial and chemical detection system under development in this project is expected to provide opportunities for environemntal monitoring in developing areas or in the field where power and computing resources are scarce.

IMAGE GALLERY

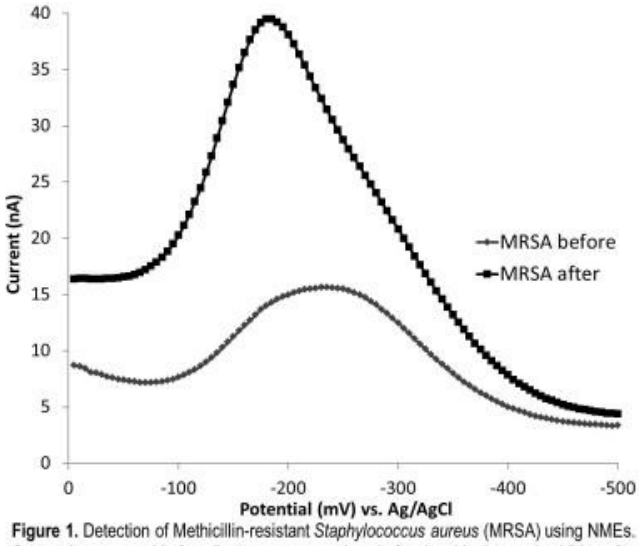


Figure 1. Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) using NMEs. Current is measured before (bottom gray curve) and after (top black curve) addition of MRSA lysate.

Example of current differences before and after addition of a bacterial sample to the NMEs.

ABSTRACT (CONTINUED FROM PAGE 1)

platform is expected to provide a sensitive, specific, and rapid sample-to-answer capability for in-flight microbial monitoring that can distinguish between related microorganisms (pathogens and non-pathogens) as well as chemical contaminants. This will dramatically enhance our ability to monitor the spacecraft environment and the health risk to the crew. Further, the project is expected to eliminate the need for sample return while significantly reducing crew time required for detection of multiple targets. Initial work will focus on the optimization of bacterial detection and identification. The platform is designed to release nucleic acids (DNA and RNA) from microorganisms without the use of harmful chemicals. Bacterial DNA or RNA is captured by bacteria-specific probe molecules that are bound to a microelectrode, and that capture event can generate a small change in the electrical current (Lam, et al. 2012. Anal. Chem. 84(1): 21-5.). This current is measured, and a determination is made whether a given microbe is present in the sample analyzed. Chemical detection can be accomplished by directly applying a sample to the microelectrode and measuring the resulting current change. This rapid microbial and chemical detection device is designed to be a low-cost, low-power platform anticipated to be operated independently of an external power source, characteristics optimal for manned spaceflight and areas where power and computing resources are scarce.

ANTICIPATED BENEFITS

To NASA unfunded & planned missions: (CONT'D)

By providing enhanced in-flight monitoring capabilities, we can reduce or eliminate the reliance on archival samples and improve our response to in-flight contamination events.

To the commercial space industry:

A device expected to provide a sensitive, specific, and rapid sample-to-answer capability for microbial and chemical detection has potential uses for evaluation of these targets in commercial space vehicles and facilities to meet standards for environmental monitoring.

To the nation:

The portability of the device and capabilities provided address existing monitoring needs for both spacecraft and terrestrial environments.