as mechanical noise to the soles of the feet, or when applied as electrical noise at the knee and to the back muscles.

SR using imperceptible stochastic electrical stimulation of the vestibular system (stochastic vestibular stimulation, SVS) applied to normal subjects has shown to improve the degree of association between the weak input periodic signals introduced via venous blood pressure receptors and the heart-rate responses. Also, application of SVS over 24 hours improves the long-term heart-rate dynamics and motor responsiveness as indicated by daytime trunk activity measurements in patients with multi-system atrophy, Parkinson's disease, or both, including patients who were unresponsive to standard therapy for Parkinson's disease. Recent studies conducted at the NASA JSC Neurosciences Laboratories showed that imperceptible SVS, when applied to normal, young, healthy subjects, leads to significantly improved balance performance during postural disturbances on unstable compliant surfaces. These studies have shown the benefit of SR noise characteristic optimization with imperceptible SVS in the frequency range of 0–30 Hz, and amplitudes of stimulation have ranged from 100 to 400 microamperes.

This work was done by Jacob Bloomberg and Millard Reschke of Johnson Space Center; Ajitkumar Mulavara and Scott Wood of USRA; Jorge Serrador of Dept. of Veterans Affairs NJ Healthcare System; Matthew Fiedler, Igor Kofman, and Brian T. Peters of Wyle; and Helen Cohen of Baylor College. For further information, contact the JSC Innovation Partnerships Office at (281) 483-3809. MSC-25013-1

Developing Physiologic Models for Emergency Medical Procedures Under Microgravity

Lyndon B. Johnson Space Center, Houston, Texas

Several technological enhancements have been made to METI's commercial Emergency Care Simulator (ECS) with regard to how microgravity affects human physiology. The ECS uses both a software-only lung simulation, and an integrated mannequin lung that uses a physical lung bag for creating chest excursions, and a digital simulation of lung mechanics and gas exchange. METI's patient simulators incorporate models of human physiology that simulate lung and chest wall mechanics, as well as pulmonary gas exchange.

Microgravity affects how O_2 and CO_2 are exchanged in the lungs. Procedures were also developed to take into affect the Glasgow Coma Scale for determining levels of consciousness by varying the ECS eye-blinking function to partially indicate the level of consciousness of the patient. In addition, the ECS was modified to provide various levels of pulses from weak and thready to hyper-dynamic to assist in assessing patient conditions from the femoral, carotid, brachial, and pedal pulse locations.

This work was done by Nigel Parker and Veronica O'Quinn of Medical Education Tech, Inc. for Johnson Space Center. Further information is contained in a TSP (see page 1). MSC-23922-1

PMA-Linked Fluorescence for Rapid Detection of Viable Bacterial Endospores

This method has applications in the pharmaceutical, food microbiology, semiconductor, and other industries requiring surface sterilization.

NASA's Jet Propulsion Laboratory, Pasadena, California

The most common approach for assessing the abundance of viable bacterial endospores is the culture-based plating method. However, culture-based approaches are heavily biased and oftentimes incompatible with upstream sample processing strategies, which make viable cells/spores uncultivable. This shortcoming highlights the need for rapid molecular diagnostic tools to assess more accurately the abundance of viable spacecraft-associated microbiota, perhaps most importantly bacterial endospores.

Propidium monoazide (PMA) has received a great deal of attention due to its ability to differentiate live, viable bacterial cells from dead ones. PMA gains access to the DNA of dead cells through compromised membranes. Once inside the cell, it intercalates and eventually covalently bonds with the double-helix structures upon photoactivation with visible light. The covalently bound DNA is significantly altered, and unavailable to downstream molecular-based manipulations and analyses. Microbiological samples can be treated with appropriate concentrations of PMA and exposed to visible light prior to undergoing total genomic DNA extraction, resulting in an extract comprised solely of DNA arising from viable cells. This ability to extract DNA selectively from living cells is extremely powerful, and bears great relevance to many microbiological arenas.

While this PMA-based selective chemistry has been applied to several polymerase chain reaction (PCR)-based molecular protocols, it has never been coupled with fluorescence *in situ* hybridization (FISH)-based microscopic methods. FISH microscopy is a powerful technique for visualizing and enumerating microorganisms present in a given sample, which relies on the ability of fluorescently labeled oligonucleotide probes to gain access to, and hybridize with, specific nucleic acid sequences within cells. Dogmatic princi-

ples suggest that by first treating a sample with PMA and covalently modifying the DNA originating from dead cells, downstream FISH-based microscopy should then enable the direct, specific visualization and enumeration of only living, viable microorganisms. An effective and efficient coupling of PMA-based chemistry with downstream FISH-microscopic methods would significantly empower the current ability to discern viable from dead microbes by direct visualization.

The basic principle of this method is that PMA penetrates only the dead cells and/or spores, due to their compromised membrane structures. Once inside the cell, PMA strongly intercalates with DNA. PMA has a photoactive azide group that allows covalent cross-linkage to DNA upon exposure to bright white light. This photoactivation results in the formation of PMA-DNA complex that renders DNA inaccessible for hybridization reaction during FISH assay. To avoid the difficulties and problems associated with current methods for determining the actual numbers of living versus dead cellular entities examined, and biases associated therewith, a novel molecular-biological protocol was developed for selective detection and enumeration of viable microbial cells. After having been subjected to the procedures described herein, the viability (live vs. dead) of bacterial cells and spores could be discerned. Following treatment with PMA, living, viable cells and spores were shown to be receptive to fluorescently labeled oligonucleotide probes, as hybridization and FISH-based microscopy was successful. Dead cells and spores, however, were not detected, as the pretreatment with PMA rendered their DNA unavailable to hybridization with the FISH-probes.

The true novelty of the technology is the coupling of a downstream, highly specific means of visualizing microbial cells and spores with a chemical pretreatment that precludes the portion of the microbial consortium that is not living (non-viable) from being detected. This results in the ability to selectively visualize and enumerate only the living cells and spores present in a given sample, in a molecular biological fashion, without the need for heavily biased cultivation-based methodologies. This novel study demonstrates that PMA penetrates only the heat-killed spores, which precludes downstream hybridization reactions in the FISH assay. This novel PMA-FISH method is an attractive tool to detect viable endospores in spacecraft-associated environments, which is of crucial importance and benefit to planetary protection practices aimed at reducing the abundance of spacecraft-borne microbial contaminants.

This work was done by Myron T. La Duc and Kasthuri Venkateswaran of Caltech, and Bidyut Mohapatra of the University of South Alabama for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

Innovative Technology Assets Management
JPL
Mail Stop 202-233
4800 Oak Grove Drive
Pasadena, CA 91109-8099
E-mail: iaoffice@jpl.nasa.gov

Refer to NPO-48040,volume and number of this NASA Tech Briefs *issue, and the page number.*

Portable Intravenous Fluid Production Device for Ground Use

This small, portable device with high output produces medical injection-grade sterile water from potable water sources.

John F. Kennedy Space Center, Florida

There are several medical conditions that require intravenous (IV) fluids. Limitations of mass, volume, storage space, shelf-life, transportation, and local resources can restrict the availability of such important fluids. These limitations are expected in long-duration space exploration missions and in remote or austere environments on Earth. Current IV fluid production requires large factory-based processes. Easy, portable, on-site production of IV fluids can eliminate these limitations. Based on experience gained in developing a device for spaceflight, a ground-use device was developed.

This design uses regular drinking water that is pumped through two filters to produce, in minutes, sterile, ultrapure water that meets the stringent quality standards of the United States Pharmacopeia for Water for Injection (Total Bacteria, Conductivity, Endotoxins, Total Organic Carbon). The device weighs 2.2 lb (1 kg) and is 10 in. long, 5 in. wide, and 3 in. high (≈25, 13, and 7.5 cm, respectively) in its storage configuration. This handheld device produces one liter of medical-grade water in 21 minutes. Total production capacity for this innovation is expected to be in the hundreds of liters.

The device contains one battery powered electric mini-pump. Alternatively, a manually powered pump can be attached and used. Drinking water enters the device from a source water bag, flows through two filters, and final sterile production water exits into a sealed, medical-grade collection bag. The collection bag contains pre-placed crystalline salts to mix with product water to form isotonic intravenous medical solutions. Alternatively, a hypertonic salt solution can be injected into a filled bag. The filled collection bag is detached from the device and is ready for use or storage. This device currently contains one collection bag, but a manifold of several pre-attached bags or replacement of single collection bags under sterile needle technique is possible for the production of multiple liters. The entire system will be flushed, sealed, and radiation-sterilized.

Operation of the device is easy and requires minimal training. Drinking water is placed into the collection bag. Inline stopcock flow valves at the source and collection bags are opened, and the mini-pump is turned on by a switch to begin fluid flow. When the collection bag is completely filled with the medical-grade water, the pump can be turned off. The pump is designed so it cannot pump air, and overfilling of the collection bag with fluid is avoided by placing an equal amount of water in the source bag. Backflow is avoided by in-