Radiation Protection Using Single-Wall Carbon Nanotube Derivatives

Stress mitigation protects DNA and other healthy cell components from the effects of radiation therapy or radiation-containing weapons.

Lyndon B. Johnson Space Center, Houston, Texas

This invention is a means of radiation protection, or cellular oxidative stress mitigation, via a sequence of quenching radical species using nano-engineered scaffolds, specifically single-wall carbon nanotubes (SWNTs) and their derivatives. The material can be used as a means of radiation protection by reducing the number of free radicals within, or nearby, organelles, cells, tissue, organs, or living organisms, thereby reducing the risk of damage to DNA and other cellular components (i.e., RNA, mitochondria, membranes, etc.) that can lead to chronic and/or acute pathologies, including but not limited to cancer, cardiovascular disease, immuno-suppression, and disorders of the central nervous system. In addition, this innovation could be used as a prophylactic or antidote for accidental radiation exposure, during high-altitude or space travel where exposure to radiation is anticipated, or to protect from exposure from deliberate terrorist or wartime use of radiation-containing weapons.

BHA and BHT are well-known food preservatives that are excellent radical scavengers. These compounds, among others, attached to SWNTs make excellent radical traps. The 4-(2-aminoethyl)-2,6-bis(1,1-dimethylethyl)phenol (amino-BHT, compound 3, Scheme 1) groups are associated with nano-engineering materials. The amino-BHT groups can be associated with SWNTs that have carboxylic acid groups via acid-base association, or via covalent attachment. The SWNTs can also have poly(ethylene glycol) (PEG) chains associated with them to enhance the solubility of the nano-engineered materials in water and buffered systems. Likewise, 4-(2-carboxyethyl)-2,6-bis(1,1-dimethylethyl)phenol (carboxy-BHT, compound 4, Scheme 2) can be associated with aminated SWNTs (i.e., SWNTs that are carboxylated, then aminated via interaction with poly(ethylene imine), again via acid base association.

One idea is to attach 2,6-di(*tert*-butyl)phenols (BHT and BHA analogs) to SWNTs, and to use these as delivery agents to quench large amounts of radicals that can be established in a cell due to oxidative stress or radiation-induced pathways. The *tert*-butyl groups are most properly named as 1,1-dimethylethyl moieties. Many other radical scavengers can be appended to the sidewalls of water-soluble SWNTs via acid-base, covalent or non-covalent (pi-pi interactions of Van der Waals interactions) functionalized protocols. In some cases, the parent pluronic-wrapped SWNT can show efficacy in this reaction as well. Two other known therapeutic radical scavengers include Lavendustin B and Amifostin. One skilled in the art can think of several permutations for derivatizing radical scavengers to SWNTs or double-wall nanotubes (DWNTs) for multi-wall nanotubes (MWNTs) where there are three or more walls predominating in a sample.

This work was done by James M. Tour, Meng Lu, Rebecca Lucente-Schultz, Ashley Leonard, Condell Dewayne Doyle, Dimitry V. Kosynkin, and Brandi Katherine Price of Rice University for Johnson Space Center.

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:

Rice University
Office of Technology Transfer MS-705
P.O. Box 1892
Houston, TX 77251-1892
Phone No.: (713) 348-6188
E-mail: techtran@rice.edu

Refer to MSC-24383-1, volume and number of this NASA Tech Briefs *issue, and the page number.*

PMA-PhyloChip DNA Microarray To Elucidate Viable Microbial Community Structure

This technology has applications in pharmaceutical and medical equipment manufacturing, and food processing.

NASA's Jet Propulsion Laboratory, Pasadena, California

Since the Viking missions in the mid-1970s, traditional culture-based methods have been used for microbial enumeration by various NASA programs. Viable microbes are of particular concern for spacecraft cleanliness, for forward contamination of extraterrestrial bodies (proliferation of microbes), and for crew health/safety (viable pathogenic microbes). However, a "true" estimation of viable microbial population and differentiation from their dead cells using the most sensitive molecular methods is a challenge, because of the stability of DNA from dead cells.

The goal of this research is to evaluate a rapid and sensitive microbial detection concept that will selectively estimate viable microbes. Nucleic acid amplification approaches such as the

polymerase chain reaction (PCR) have shown promise for reducing time to detection for a wide range of applications. The proposed method is based on the use of a fluorescent DNA intercalating agent, propidium monoazide (PMA), which can only penetrate the membrane of dead cells. The PMA-quenched reaction mixtures can be screened, where only the DNA from live cells will be available for subsequent PCR reaction and microarray detection, and be identified as part of the viable microbial community. An additional advantage of the proposed rapid method is that it will detect viable microbes and differentiate from dead cells in only a few hours, as opposed to less comprehensive culture-based assays, which take days to complete. This novel combination approach is called the PMA-Microarray method.

DNA intercalating agents such as PMA have previously been used to selectively distinguish between viable and dead bacterial cells. Once in the cell, the dye intercalates with the DNA and, upon photolysis under visible light, produces stable DNA adducts. DNA cross-linked in this way is unavailable for PCR. Environmental samples suspected of containing a mixture of live and dead microbial cells/spores will be treated with PMA, and then incubated in the dark. Thereafter, the sample is exposed to visible light for five minutes, so that the DNA from dead cells will be cross-linked. Following this PMA treatment step, the sample is concentrated by centrifugation and washed (to remove excessive PMA) before DNA is extracted. The 16S rRNA gene fragments will be amplified by PCR to screen the total microbial community using PhyloChip DNA microarray analysis. This approach will detect only the viable microbial community since the PMA intercalated DNA from dead cells would be unavailable for PCR amplification. The total detection time including PCR reaction for low biomass samples will be a few hours.

Numerous markets may use this technology. The food industry uses spore detection to validate new alternative food processing technologies, sterility, and quality. Pharmaceutical and medical equipment companies also detect spores as a marker for sterility. This system can be used for validating sterilization processes, water treatment systems, and in various public health and homeland security applications.

This work was done by Kasthuri J. Venkateswaran and Christina N. Stam of Caltech, and Gary L. Andersen and Todd DeSantis of Lawrence Berkeley National Laboratory 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-47112, volume and number of this NASA Tech Briefs *issue, and the page number.*