From the cover: “Secretory proteins of salivary glands, whose expression is regulated by cyclic AMP-PKA signaling, respond to microgravity. A component of the signaling pathway, RII, may serve as a stress biomarker.” M.I. Mednieks et al., p. 2.
GENERAL INFORMATION

*Gravitational and Space Research* (ISSN 2332-7774) is a journal devoted to research in gravitational and space sciences. It is published by the American Society for Gravitational and Space Research, a non-profit organization whose members share a common goal of furthering the understanding of the effects of gravity and the use of the unique environment of spaceflight for research. *Gravitational and Space Research* is overseen by a steering committee consisting of the Publications Committee, the Editor, the President, and the Secretary-Treasurer of the ASGSR.

The American Society for Gravitational and Space Biology was created in 1984 and became *The American Society for Gravitational and Space Research* in 2012 to provide an avenue for scientists interested in gravitational and space biology to share information and join together to speak with a united voice in support of this field of science. The effects of gravity have been acknowledged since Galileo’s time, but only since the 1970s has gravitational research begun to attract attention. With the birth of the space age, the opportunity for experimentation over the full spectrum of gravity finally became a reality, and a new environment and research tool became available to probe gravitational phenomena and expand scientific knowledge. Space and spaceflight introduced new questions about space radiation and the physiological and psychological effects of the artificial environment of spacecraft.

The objectives of ASGSR are:

- To promote research, education, training, and development in the areas of gravitational and space research and to apply the knowledge gained to a better understanding of the effect of gravity and space environmental factors on the flora and fauna of Earth.
- To disseminate information on gravitational and space research and the application of this research to the solution of terrestrial and space problems.
- To provide a forum for communication among professionals in academia, government, business, and other segments of society involved in gravitational and space research and application.
- To promote the study of concepts and the implementation of programs that can achieve these ends and further the advancement and welfare of humankind.

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Gravitational and Space Research

Instructions for Authors

Brief Overview:

The journal of the American Society for Gravitational and Space Research (ASGSR), *Gravitational and Space Research*, publishes quality, peer reviewed manuscripts in several categories. Manuscripts should be self-contained, and all conclusions substantiated and supported by results in the form of figures and/or tables. Authors are held to standards of writing (American English) for clarity and material appropriate for the *Gravitational and Space Research* (GSR) journal. Subject matter can include any topic within the following broad categories: the impact of gravity and changes in the gravity vector on biology, astrobiology, spaceflight and planetary analog environment research, advanced life support (ALS), biophysics, radiation biology, human-tended spaceflight, satellite payloads, suborbital research, parabolic flight, sounding rockets, high altitude balloons, hardware engineering and development, acceleration in altered gravity environments, combustion science, complex fluids, fluid physics, fundamental physics, and materials science. In addition, research wholly dedicated to terrestrial explorations of the impact of gravity and to changes in the gravity vector is welcome. The categories of papers include Short Communication, Methods, Research, Hypothesis, and Review. We are also open to publishing Educational Outreach papers, and can embed links to additional materials, including videos and large databases.

I. Short Communications

Short communications are submissions typically 2 - 3 pages in length (1000 – 2500 words, excluding references). These manuscripts are generally comprised of preliminary data supporting work in progress, or a brief report to showcase a stand-alone facet of a larger project.

II. Methods Papers

Methods papers are manuscripts typically 3 - 6 pages in length and, although there is no strict limitation to size, a reviewer may address extremes of brevity or length as appropriate to conveying the information presented. These manuscripts are primarily comprised of data and protocols that support the design and execution of experiments in any of the categories defined in the overview. The manuscripts should contain sufficient detail to enable a reader to replicate the protocol. Figures should include illustrations of procedures and set-up, and should include data that verify the efficacy of the procedures.

III. Research Papers

Research papers are manuscripts of typically 8 - 15 pages in length. These manuscripts present original research of interest to the gravitational and space research community.

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Review articles are typically 10 - 15 pages in length. These manuscripts are often solicited from symposium speakers at the annual ASGSR meeting, but they are not limited to those solicitations. Any author may approach the editorial board with a suggestion or request to submit a review article, which will be peer-reviewed as any other paper.
Detailed Instructions

Format

The same basic format is used for each type of article. Consult a current issue of *Gravitational and Space Research*, as well as the instructions below, for guidance on formatting, organizing, and preparing references, figures, tables, and legends. An article must have a brief abstract (less than 250 words) that summarizes the principal conclusions of the paper. A Template in Microsoft Word can be downloaded from the Journal website (http://GravitationalAndSpaceResearch.org) that conforms to the general Journal requirements. Alternatively, the manuscript can be formatted for submission along the following guidelines (see also Arrangement section below):

**Text:** Single spaced, Times New Roman 11, paragraphs indent 0.25 inch. Do not insert line space after paragraph. Do not use page breaks.

**Citations:** In text by (Author, year) mode; details below.

**Headings:** HEADING LEVEL ONE; Bold, all capital.

**Subheadings:** Heading level two; Bold, sentence case.

**Subheadings:** Heading level three; Italic, non-bold, sentence case.

**Reference section:** Must comply with format below, each citation separated by line space.

Length

Length is dependent on the type of manuscript being submitted; see overviews above for specifics. One page of a GSR article is typically comprised of 750 – 950 words (depending on the number of figures) and articles range from 2-3 pages for Short Communications, to 15 pages for full Research Papers and Review Articles.

Abbreviations

- Do not use abbreviations other than those that are standard for international usage.
- Use SI units as far as possible.
- Use $g$ (italicized) for unit gravity, to distinguish it from the standard abbreviation $g$ (not italicized) for gram.
- Any acronyms that are used in the manuscript must be defined at first mention.

Arrangement

Arrange the manuscript in the following order, with all pages numbered consecutively in the footer of the lower right corner. The last name of the first author should precede each page number. A Template in Microsoft Word can be downloaded from the Journal website (http://GravitationalAndSpaceResearch.org) that conforms to the general Journal requirements.

**Running Title:** Title case. To be used as a header; it is not to exceed 60 characters, including spaces.

**Full Title:** Title case. Use a descriptive title, but do not exceed 200 characters, including spaces.

**All Authors:** Provide full names and affiliations (institution, city, state) in the order in which they are to be listed. Use numerical superscripts to identify affiliations.

**Corresponding Author:** Full name, affiliation and address, e-mail, telephone number.
Abstract: Summarize the principal approach and conclusions of the paper (not to exceed 250 words).

Body of paper: For Research Papers, the body of the paper should be arranged into subsections for Introduction, Materials and Methods, Results, and Discussion. Review Papers should be organized in a manner appropriate to the subject. Methods papers should include a short Introduction and also a Discussion of the application addressing the significance of the method being described.

References and Citations: Cite each reference in the text by author(s) name(s) and the publication date: Examples: Smith, 1989 (one author); Smith and Jones, 2001 (two authors); Smith et al., 2010 (more than two authors). An EndNote® Style file can be downloaded at the GSR website (http://GravitationalAndSpaceResearch.org).

- Alphabetize the reference list by authors' last names.
- List only published or in-press articles. Unpublished results, including personal communications and submitted manuscripts, should be cited as such in the text.
- References formatted as follows: last name(s) of author(s), followed by initials with no space; year of publication in parentheses; article title in sentence case, followed by a period; journal title (unabbreviated and italicized), followed by volume number in bold, issue number in parenthesis (if applicable), a colon, a space, and page numbers.

Four examples below:

**Journal Article:**

**Book:**

**Edited Book:**

**Conference Proceedings:**

Figure legends: Provide full, descriptive figure legends for each figure; each figure legend should be composed of a short descriptive title, followed by a description of the material illustrated in the figure. Figure legends should provide any information key to understanding the material presented in the figure.

Figures: Submit Figures as individual graphics files (TIF, BMP, or high resolution JPG). Resolution must be at least 300dpi for photographs and 600dpi for line graphics. Include Figure number in the file name when uploaded. For publication, Figures will be formatted to either one column width (3 inches / 7.6cm) or across two columns (6.3 inches / 16cm). Note that all lettering and numbers within Figures must be at least font size 8 when reduced to publication width. This restriction includes numbers and axis information in graphs as well. Authors are encouraged to test size their Figures in these two dimensions to ensure compliance, as Figures which contain illegible annotations will be returned for
reconfiguration.

- Number Figures consecutively as they are used in the text. Use Figure 1, i.e., capitalize and the full word “Figure” (do not use Fig. 1).
- The first time a Figure is discussed, refer to it actively rather than parenthetically.
- Provide enough information in the Figure Legend such that the reader can understand the Figure without significant input from the text.
- Designate Figure sections with letters and explain all symbols and abbreviations that are used in the Figure.

Tables: Provide at the end of the manuscript.

- Number Tables consecutively as they are used in the text.
- The first time a Table is discussed refer to it actively, rather than parenthetically.
- Give each Table a concise title, followed by a legend that makes the general meaning of the Table comprehensible without reference to the text.
- Tables should be constructed in Word or Excel with the general format below. Font size 10 should be used for the primary Table text, but font size 8 may be used for footnotes or annotations.

<table>
<thead>
<tr>
<th>Table 1. Atmospheric pressure relative to altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pressure (kPa)</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>101 – 70</td>
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<tr>
<td>70 – 50</td>
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<td>50 – 30</td>
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<td>30 – 5</td>
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</tbody>
</table>

Footnotes as necessary

Manuscript Peer Review and Preparation of Final Version

Prior to publication, manuscripts are reviewed by the managing editor who is assigned to an author’s article, and by two to three external scientific reviewers. Manuscripts submitted without complying with submission requirements may be returned for format changes before being accepted for review.

Once a manuscript is accepted, the manuscript is reviewed internally for copyediting, and then sent to the publishing editor. Page proofs are provided to the authors for review prior to publication.

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Authorship Statement

Authorship of articles implies that an individual has made a substantial contribution to the article, both in terms of the design of the study or collection/evaluation of data and with regard to the intellectual content of the manuscript.
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The general requirements for informed consent conform to guidelines and requirements outlined by the National Science Foundation (http://www.nsf.gov/bfa/dias/policy/docs/45cfr690.pdf) and Health and Human services (http://www.hhs.gov/ohrp/policy/consent/). No investigator may involve a human being as a subject in research covered by this policy unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the
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In seeking informed consent the following information shall be provided to each subject:

- A statement that the study involves research, an explanation of the purposes of the research, and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;
- A description of any reasonably foreseeable risks or discomforts to the subject;
- A description of any benefits to the subject or to others which may reasonably be expected from the research;
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;
- A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained;
- For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained;
- An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and
- A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.
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Salivary Gland Protein Expression after Bion-M1 and Space Shuttle STS-135 Missions

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¹Department of Oral Health and Diagnostic Sciences; ²Department of Craniofacial Sciences, University of Connecticut School of Dental Medicine, Farmington, CT

ABSTRACT

Secretory proteins produced by salivary glands are stored in granules and released into saliva. Rodent salivary glands are a reliable experimental model because they are morphologically and functionally similar to those of humans. To determine if the effects of microgravity on secretory proteins are increased on extended flights, their expression in mouse parotid glands, morphological, immunocytochemical, and biochemical/molecular methods were employed. Acinar cells of STS-135 (13 day) and Bion-M1 (30 day) flight animals showed an increase of autophagy and apoptosis, while duct cells contained vacuoles with endocytosed proteins. In STS-135, decreases were seen in the regulatory subunit of type II protein kinase A (RII) by Western blotting, and demilune cell and parotid protein (DCPP) and α-amylase (p<0.01) by immunogold labeling, while proline-rich proteins (PRPs, p<0.001) and parotid secretory protein (PSP, p<0.05) were increased. These results suggest microgravity effects on secretion are function-dependent. Microarray analyses showed significant changes in the expression of a number of genes, including components of the cyclic-3',5'-adenosine monophosphate (cyclic AMP) signaling pathway. Compared to habitat ground controls, mice from both flights exhibited altered expression of cyclic AMP-specific phosphodiesterases, adenylyl cyclase isoforms, and several A-kinase anchoring proteins. Bion-M1 flight mice showed increases in gene expression for lysozyme and amylase, a decrease in PRPs, and RII expression was unchanged from control values. Secretory protein expression is altered by travel in space, representing a reversible adjustment to microgravity conditions. Ultimately, the goal is to develop a test kit using saliva — an easily obtained body fluid — to assess the physiologic effects of travel in space.

INTRODUCTION

Background

Understanding the effects of weightlessness during travel in space is important in order to institute countermeasures if the findings are
detrimental. Catecholamine hormone-controlled cellular events are altered during spaceflight (Mednieks et al., 1998; Mednieks et al., 2000; Mednieks et al., 2014), indicating an environmental effect or a response to microgravity. In salivary glands, β-adrenergically regulated responses modify exocytosis and protein secretion (Bdolah and Schramm, 1965; Butcher and Putney, 1980; Mednieks and Hand, 1982; Horio et al., 1984). Protein expression, mediated via cyclic AMP pathways, is particularly affected and may serve as an index of physiological or disease-related changes (Gold et al., 2013) [e.g., in diabetes (Szczepanski et al., 1998; Mednieks et al., 2009)], as well as responses to mechanical (Burke et al., 2002) or environmental stimuli (Beavo and Brunton, 2002).

Figure 1 is a diagram of the components of exocytotic stimulus pathways with the β-adrenergic receptor (βAR) activating adenylate cyclase (AC), formation of cyclic AMP [with phosphodiesterase (PDE) to lower intracellular levels], activation of cyclic AMP-dependent protein kinase (PKA) by subunit dissociation, and the eventual nuclear reactivity of the type II regulatory subunit (RII) via the action of DNA binding A-kinase anchor proteins (AKAPS) (Dodge et al., 2001; Herberg et al., 2000). The metabolic interplay of these components provides an internal stability to environmental changes at the cellular level of organization. The individual molecular components of this pathway are well-studied (Daniel et al., 1998; Gold et al., 2013) and provide a series of biomarkers for biochemically measuring stress responses. Serum levels of these components are difficult to analyze and dependent on a variety of internal variations, and the collection of blood and urine for bio-fluid analysis is not convenient. However, the RII is secreted in saliva (Mednieks and Hand, 1984) and is an easily measurable biochemical indicator of environmental stress.

Numerous other salivary secretory proteins with known function may be indices of metabolic pathways and associated physiologic or pathologic functions (Mandel, 1993; Ruhl, 2012). Among those considered in this study are alpha-amylase (α-amylase) (Valdez and Fox, 1991), the proline-rich proteins (PRPs) (Mehansho et al., 1985; Carlson et al., 1991), demilune cell and parotid protein (DCPP) (Bekhor et al., 1994), and parotid secretory protein (PSP) (Owerbach and Hjörth, 1980; Ball et al., 2003). Microarray analysis of mouse salivary gene expression was carried out comparing flight with control data (Mednieks et al., 2014). By considering this initial array of secretory protein responses, a number of criteria can be established to assess the effects of the length of microgravity exposure and eventual transience or permanence of these effects upon return to Earth.

Objectives

Human and rodent salivary glands are morphologically and functionally similar. In both species, secretory proteins produced by salivary glands are stored in granules and released into saliva where they can be measured. The mouse model is, therefore, appropriate for studies of stimulated secretion. This system avoids the difficulties associated with obtaining tissue from human subjects and saliva from test animals. Our objectives, therefore, are: 1) To study the effects of weightlessness at the tissue, cell, and molecular level; 2) to identify specific molecular mechanisms affected by space travel; 3) to propose the design of a simple, economic device to measure changes in salivary proteins that can be used to monitor the physiological status of astronauts in space, or patients in Earth-based clinics.

METHODS

Animals and Space Flight / Ground Control Conditions

Thirty C57Bl/6J adult female mice, nine weeks old at launch, were housed in Animal Enclosure Modules (AEMs) and flown on the space shuttle Atlantis STS-135 mission, launched from Kennedy Space Center on July 8, 2011, with landing on July 21, 2011. Fifteen mice housed in AEMs served as ground controls. Fifteen additional control mice were housed under standard vivarium conditions (for experiment details see Gridley et al., 2013). The flight mice and AEM ground control mice were fed identical diets [NASA rodent food bars (Sun et al., 2014)] and had access to water ad libitum. The vivarium control mice were fed pelleted rodent chow. Tissues from seven of the flight mice were
obtained within 2-5 hours after landing. Tissues from the vivarium control mice were obtained one day prior to landing, and tissues from the AEM control mice were obtained 48 hours after landing. All animal procedures were approved by the Institutional Animal Care and Use Committee at the National Aeronautics and Space Administration (NASA), and conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Mice from the Bion-M1 mission were C57BL/6N specific pathogen-free males, 19-20 weeks old at launch on April 19, 2013. Samples were obtained from six flight mice between 13-16.5 hours after landing on May 19, 2013, and from eight vivarium control mice immediately afterwards. Samples were also obtained from seven asynchronous control mice, housed two months after the mission in flight habitats for 30 days under environmental conditions simulating the flight conditions, and seven asynchronous vivarium control mice. The flight mice and the asynchronous habitat control mice were fed a paste diet based on standard rodent chow, with water and casein added as a gelling agent. Vivarium control mice were fed pelleted rodent chow. Details of the experimental conditions can be found in Andreev-Andrievsky et al. (2014). All animal procedures were approved by the Institutional Animal Care and Use Committee of Moscow State University Institute of Mitoengineering and the Biomedical Ethics Commission of the Institute for Biomedical Problems, and conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Tissue Collection

After euthanasia, the salivary glands were excised and placed in cold phosphate buffered saline (PBS). A portion of each gland was rapidly frozen in a protease inhibitor mixture for protein assays and in RNAlater or Allprotect (Qiagen, Hilden, Germany) for RNA analyses. The remaining tissues were minced and fixed in 2.5% glutaraldehyde-2% paraformaldehyde for electron microscopy, or 4% paraformaldehyde for immunoctochemistry. The samples were shipped by overnight express to the University of Connecticut Health Center.

Secretory Proteins and Immunological Reagents

The specific secretory proteins studied were α-amylase, PSP, DCPP, PRPs, the regulatory subunit of type II protein kinase A (PKA RII) (regulatory protein), salivary androgen binding protein alpha (SABPα), epidermal growth factor (EGF), and nerve growth factor (NGF). Antibodies used were: anti-RII (Szczepanski et al., 1998; Mednieks et al., 2008), anti-α-amylase and anti-EGF (Sigma-Aldrich, St. Louis, MO), anti-PSP (Ball et al., 1988; L. Mirels, unpublished), anti-PRPs (D.M. Carlson, unpublished), anti-DCPP and anti-SABPα (L. Mirels, unpublished), and anti-NGF (Santa Cruz Biotechnology, Dallas, TX).

Transmission Electron Microscopy (TEM)

Parotid glands were postfixed in 1% osmium tetroxide, treated with 1% aqueous uranyl acetate, dehydrated in ethanol, and embedded in epoxy resin following standard procedures (Hand, 1995). Thin sections were stained with uranyl acetate and lead citrate, and examined in a Hitachi H7650 TEM.

Immunogold Labeling TEM

Tissues were embedded in LR Gold resin (London Resin Co., Ltd., Electron Microscopy Sciences, Hatfield, PA) at -20°C. Thin sections were collected on Formvar-coated nickel grids, treated with 1% BSA/5% NGS in PBS to block nonspecific binding, and incubated with primary antibody overnight at 4°C. Bound antibodies were visualized with gold-labeled goat anti-rabbit IgG (Aurion, Electron Microscopy Sciences) or gold-labeled protein-A (BB International, Ted Pella, Redding, CA). The sections were stained with uranyl acetate and lead citrate and examined in the TEM. Digital images were taken at 10,000X using an AMT XR41C camera (Advanced Microscopy Techniques, Woburn, MA).

Quantification of immunogold labeling was done on the TEM images. Using Photoshop (CS2 v. 9.0.2 or CS4 Extended v. 11.0.2), a grid pattern with a separation between grid lines corresponding to 0.5 µm was superimposed on the image. Grid intersections lying over the organelle
of interest were counted and divided by 4 to estimate area in \( \mu m^2 \). Gold particles labeling the organelle were counted and divided by the area to give a labeling density (gold particles/\( \mu m^2 \)). Significance was assessed using ANOVA and a two-tailed T-test.

**Electrophoresis and Western Blotting**

Tissue samples were homogenized in PBS containing 12.5 mM benzamidine and mM phenylmethyl sulfonyl fluoride. The homogenate was centrifuged at 10,000xG for 10 min at 4°C and the supernatant was collected. A light microscope smear was made of the pellet to ascertain effective homogenization of cells and organelles. Concentration of total proteins in the soluble fraction was determined by densitometric analysis of Ponceau S dye intensity on slot blots, compared to those of standards of known protein concentration. The sample proteins were adjusted to identical protein concentrations per lane and subjected to SDS–polyacrylamide gel electrophoresis (PAGE) using pre-cast 10% polyacrylamide gels. The proteins were electrotransferred to nitrocellulose membranes, stained with Ponceau S, scanned, and digitized to visualize electrophoretic banding patterns. The membranes were then washed to remove the dye, blocked with a 3% milk solution in PBS - 0.01% Tween20, and incubated with specific primary antibodies. Horseradish peroxidase-conjugated secondary antibodies were applied, and the Western blot developed with 4-chloro-1-naphthol-H\(_2\)O\(_2\) solution, or Vector NovaRED substrate (Vector Laboratories, Burlingame, CA). Protein concentrations and protein banding pattern profiles were determined by scanning and measuring the digitized images using ImageJ (v. 1.43u or 1.48; Public domain) software and compared to the densities of standard proteins of known concentration. A standard curve was prepared for each experiment. Average values obtained by scanning of duplicate lanes were used for comparisons among the groups. Due to the small sample size, additional Western blotting was carried out using a slot blotting method (HYBRI-SLOT™ MANIFOLD, Life Technologies Inc., Gaithersburg, MD). Duplicate samples were on the two slot blot lanes of the same membrane; antibody incubations, color development, and digitized sample analysis were carried out as described above for the electrophoretically separated protein samples.

**Microarray Analyses**

Individual tissue samples — eight control and seven flight from STS-135, and seven control and five flight from Bion-M1 — were submitted for RNA isolation and microarray analyses by Phalanx Biotech Group (San Diego/Belmont, CA) using Mouse OneArray 2 Gene Expression Profiling. All samples had high (>6) Agilent 6000 Nano Assay RNA integrity (RIN) numbers. Information about the mouse microarray can be found at http://www.phalanxbiotech.com/products/MOA.php (accessed 5/25/15), and their gene expression service at http://www.phalanxbiotech.com/services/service_genome.php (accessed 5/25/15).

**RESULTS**

**Morphology and Immunocytochemistry**

The flight mice from both STS-135 and Bion-M1 appeared healthy upon landing. The flight mice and the AEM (habitat) control mice from STS-135 mission lost an average of 2.3 and 1.3 grams of body weight, respectively (Table 1). The flight mice and habitat control mice from the Bion-M1 mission both gained weight, 2.6 and 2.4 grams, respectively. There were no significant differences between the body weights of the flight mice and their respective habitat or vivarium controls, thus indicating a similar nutritional status.

The morphology of a parotid acinar cell of a STS-135 flight mouse is shown in Figure 2, Panel A. The cells have a basally located nucleus, abundant rough endoplasmic reticulum, a prominent Golgi complex, and numerous secretory granules stored in the apical cytoplasm. The secretory granules typically have a variable density, with a peripheral dense region and a lighter central region. Sometimes a dense area is present in the middle of the light region. The apical surface of the cells faces the central lumen of the acinus, and finger-like extensions of the lumen — intercellular canaliculi — are located along the lateral sides of the cells. Junctional complexes join the cells at the apical ends of the lateral intercellular spaces and seal the intercellular canaliculi from the intercellular spaces.
Table 1. Body Weights of STS-135 and Bion-M1 Mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Wt. (g) ± SEM (n)</th>
<th>Final Body Wt. (g) ± SEM (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STS-135</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight</td>
<td>20.7 ± 0.40 (7)</td>
<td>18.4 ± 0.52 (7)</td>
</tr>
<tr>
<td>AEM Ground Control</td>
<td>20.7 ± 0.31 (15)</td>
<td>19.4 ± 0.33 (15)</td>
</tr>
<tr>
<td>Vivarium Ground Control</td>
<td>---</td>
<td>19.7 ± 0.34 (15)</td>
</tr>
<tr>
<td><strong>Bion-M1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight</td>
<td>26.8 ± 0.55 (6)</td>
<td>29.4 ± 1.73 (6)</td>
</tr>
<tr>
<td>Synchronous Vivarium Ground Control</td>
<td>29.2 ± 0.44 (8)</td>
<td>28.7 ± 0.30 (8)</td>
</tr>
<tr>
<td>Asynchronous Habitat Ground Control</td>
<td>27.3 ± 1.15 (7)</td>
<td>29.7 ± 0.83 (7)</td>
</tr>
<tr>
<td>Asynchronous Vivarium Ground Control</td>
<td>26.7 ± 0.78 (7)</td>
<td>28.9 ± 1.03 (7)</td>
</tr>
</tbody>
</table>

Figure 1. Cyclic AMP-PKA signaling pathways of regulated exocytosis in cell compartments. Schematic representation of cell surface beta-adrenergic receptor stimulation, signal transduction, cyclic AMP second messenger cytoplasmic and nuclear response. βAR, beta-adrenergic receptor; Gs, heterotrimeric G protein; AC, adenylate cyclase; PDE, phosphodiesterase; AKAP, A-kinase anchor protein; RII, type II PKA regulatory subunit; C, PKA catalytic subunit; ATF1, activating transcription factor 1; CREB, cyclic AMP response element binding protein; CREM, cyclic AMP responsive element modulator. The large blue sphere represents a secretory granule along a path to fuse with the cell membrane and to empty its contents of secretory proteins into the lumen.
space. The basic structure of the acinar cells of STS-135 and Bion-M1 flight mice were similar, and not different from that of habitat or vivarium control mice.

There were, however, some specific changes observed in the glands of flight animals. Figure 2, Panel B, shows an intercalated duct of a STS-135 flight mouse. Intercalated ducts are the first component of the duct system connecting the acini with the striated ducts, which are the major ductal component and are involved in electrolyte reabsorption and secretion. The cells of both intercalated and striated ducts — especially in STS-135 samples, but also in Bion-M1 samples — had large apical vacuoles containing acinar secretory proteins endocytosed from the ductal lumen. The inset in Panel B shows immunogold labeling of a vacuole in a striated duct cell for PSP, an acinar secretory protein. The acinar cells of mice from both flights also showed an increased number of autophagic vacuoles and an increase in the number of apoptotic cells was observed. Panel C shows autophagic vacuoles in a Bion-M1 flight mouse containing secretory proteins and other organelles undergoing degradation. Gold particles indicating the presence of PSP, an acinar secretory protein, label the content of one autophagic vacuole and other secretory granules in the cell. Panel D illustrates two apoptotic acinar cells engulfed by a macrophage in a Bion-M1 flight mouse. The structure of the surrounding acinar cells appears normal.

Quantitative immunogold labeling was done to assess secretory protein expression in acinar cells of STS-135 and Bion-M1 flight and habitat control mice. Figure 3, Panels A and B, shows PRP labeling of secretory granules in STS-135 flight and AEM ground control samples, respectively. As shown in the micrographs, the labeling density (gold particles/µm²) of the flight granules was greater than that of the control granules, indicating greater expression of PRP in the flight mice. Panels C and D show amylase labeling in Bion-M1 flight and habitat ground control samples, respectively, demonstrating increased amylase expression in flight mice compared to habitat controls. A summary of the immunogold labeling data for flight mice, and their respective habitat controls from both STS-135 and Bion-M1, is shown in Table 2 and Figure 4. The longer flight duration of Bion-M1 resulted in some specific differences in secretory protein expression. Amylase expression was decreased in STS-135 flight samples, but increased in Bion-M1 flight samples. Expression of PSP was slightly, but significantly, increased in both flights. Expression of PRP was increased in STS-135 flight samples, but slightly decreased in Bion-M1 flight samples. On the other hand, expression of DCPP was slightly decreased in both STS-135 and Bion-M1 flight samples, and PKA RII expression was essentially unchanged from habitat controls. Table 2 also shows results of immunogold labeling of submandibular glands of Bion-M1 flight and habitat control mice. Expression of the acinar cell proteins SABPα and PRP, and the granular duct cell proteins EGF and NGF, was significantly increased in flight mice compared to the controls.

**Electrophoresis and Western Blotting**

The Bion-M1 Western blotting results showed no difference between RII in Bion-M1 flight and vivarium control mice, seen in Figure 5, Panel A. Electrophoretic banding patterns of the flight and control animals, shown in Figure 5, Panel B, were virtually identical. Similar electrophoretic banding patterns and densitometry profiles were seen in STS-135. However, as shown in the top profile of Figure 6, Panel A, the Western blotting RII band of the STS-135 flight samples was significantly smaller than the corresponding band in either vivarium or habitat controls, middle and bottom profiles, respectively. These results indicate that on the shorter, STS-135 flight the expression of RII was decreased, while in the longer Bion-M1 flight an apparent stabilization or adjustment to the microgravity environment had occurred and the RII levels were not different from those of either of the controls. Both Bion-M1 and STS-135 flight and both control samples show a significant, faster-moving fragment (Rᵢᵣ) that is recognized by the anti-RII antibody. Protease inhibitors were used and samples frozen rapidly after dissection, nevertheless the faster moving component seen in the Western blot (WB) may be due to proteolysis, as may the relatively high backgrounds. Table 3 shows the ratios of the flight Rᵢᵣ to total proteins are not significantly different for either the STS-135 or Bion-M1 samples compared to controls. The decrease, therefore, in RII for the STS-135
Figure 2. Ultrastructure of parotid glands of flight mice. Panel A, STS-135: Acinar cells have basally located nuclei [N (binucleate cells are common)], a prominent Golgi complex (G), and abundant apically located secretory granules (SG). Intercellular canaliculus (IC). Scale bar = 2 µm. Panel B, STS-135: Intercalated duct. The duct cells have apical vacuoles (arrows) containing material endocytosed from the lumen (L). Scale bar = 2 µm. The inset shows a similar vacuole in a striated duct cell immunogold labeled for PSP, an acinar secretory protein. Scale bar = 0.5 µm. Panel C, Bion-M1: A recently formed autophagic vacuole (AV1) containing secretory proteins labeled for PSP is present in an acinar cell. An older autophagic vacuole (AV2) contains remnants of secretory granules and other organelles, but shows little labeling. Secretory granule (SG); nucleus (N). Scale bar = 1 µm. Panel D, Bion-M1: Apoptotic acinar cells (Apop) phagocytosed by a macrophage (arrowheads). Myoepithelial cell (MEC). Scale bar = 2 µm.
flight may be due to flight conditions rather than to increased degradation. However, the ratio of RII/protein in flight samples compared with both vivarium and habitat control samples in STS-135 is significantly lower than that of Bion-M1. These results are consistent with those from immunocytochemistry experiments and microarray analyses. Figure 6, Panel B, shows that the expression of α-amylase is significantly reduced in the flight parotid, and also in the habitat control when compared to vivarium controls.

**Microarray Analyses**

Changes in the expression of selected genes associated with cyclic AMP signaling pathways in the flight animals compared with habitat control mice are shown in Figure 7. The flight duration (STS-135, 13 days; Bion-M1, 30 days) had a significant effect on the expression of most of these genes. The expression of the type II PKA regulatory subunit (Prkar2a) was decreased in STS-135 flight mice, but was near control levels in Bion-M1 flight mice. Phosphodiesterase 4a (Pde4a) also was decreased, more so in Bion-M1 flight mice than STS-135 flight mice. The two flights showed opposite effects on the expression of A-kinase anchor protein 13 (Akap13) and adrenergic receptor beta 2 (Adrb2); the former increased in STS-135 mice and decreased in Bion-M1 mice, whereas the latter decreased in STS-135 mice and increased in Bion-M1 mice. Adenylate

**Table 2. Quantitative Immunogold Labeling of STS-135 and Bion-M1 Salivary Glands (Gold Particles/μm² ± SEM).**

<table>
<thead>
<tr>
<th>Bion-M1</th>
<th>Flight</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>15.3 ± 1.11</td>
<td>10.3 ± 0.69</td>
</tr>
<tr>
<td>RII</td>
<td>10.1 ± 0.47</td>
<td>11.3 ± 0.49</td>
</tr>
<tr>
<td>PSP</td>
<td>37.4 ± 1.17</td>
<td>30.6 ± 1.76</td>
</tr>
<tr>
<td>PRP</td>
<td>22.5 ± 1.61</td>
<td>24.8 ± 2.08</td>
</tr>
<tr>
<td>DCPP</td>
<td>23.1 ± 0.95</td>
<td>25.2 ± 1.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STS-135</th>
<th>Flight</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>13.9 ± 0.58</td>
<td>17.0 ± 0.76</td>
</tr>
<tr>
<td>RII</td>
<td>11.9 ± 0.36</td>
<td>12.1 ± 0.47</td>
</tr>
<tr>
<td>PSP</td>
<td>16.5 ± 0.35</td>
<td>15.2 ± 0.51</td>
</tr>
<tr>
<td>PRP</td>
<td>21.2 ± 0.87</td>
<td>17.2 ± 0.67</td>
</tr>
<tr>
<td>DCPP</td>
<td>12.5 ± 0.45</td>
<td>14.1 ± 0.75</td>
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<table>
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<tr>
<th>Bion-M1</th>
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<tr>
<td>Submandibular</td>
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</tr>
<tr>
<td>SABPα</td>
<td>5.30 ± 0.27</td>
<td>2.40 ± 0.13</td>
</tr>
<tr>
<td>PRP</td>
<td>6.36 ± 0.21</td>
<td>4.73 ± 0.35</td>
</tr>
<tr>
<td>EGF</td>
<td>32.5 ± 0.60</td>
<td>20.4 ± 1.09</td>
</tr>
<tr>
<td>NGF</td>
<td>8.78 ± 0.41</td>
<td>5.52 ± 0.37</td>
</tr>
</tbody>
</table>

Significantly different from habitat: * p<0.05; § p<0.01; ∞ p<0.001
Figure 3. Immunogold labeling of parotid acinar cell secretory granules. Panel A, STS-135: Flight, PRPs. Panel B, STS-135: AEM ground control, PRPs. Panel C, Bion-M1: Flight, amylase. Panel D, Bion-M1: Habitat ground control, amylase. Intercellular canaliculus (IC); mitochondrion (M). Scale bar = 0.5 μm.
Figure 4. Morphometric analysis of immunogold labeling of parotid secretory proteins. Percent change in STS-135 (light bars) and Bion-M1 (dark bars) flight mice from habitat controls.

Figure 5. Electrophoresis, Western blotting, and densitometry of parotid proteins. Panel A, Bion-M1 control and flight parotid soluble proteins. The lanes are molecular size marker (M), flight (F), and vivarium control (C). The two bands on the Western blot (WB) are at the mobility of RII and an RII fragment (Rfr), respectively. Panel B, Densitometry of the banding pattern profiles. Vivarium control (light gray curve) superimposed with the flight (darker curve) protein electrophoretic pattern. The ordinate shows integrated density (ID) values and the abscissa shows the calculated molecular size in kilo Dalton (kD) units.
Figure 6. Densitometric analysis of polyacrylamide gel electrophoretic protein separation and anti-RII and anti-α-amylase reactivity in STS-135 mice. Panel A: Top panel, flight; middle panel, vivarium; and bottom panel, habitat parotid gland samples. The lighter curve represents the protein profile; the shaded area is the reactivity to anti-RII antibody. The ordinate axes are integrated density values of the proteins on the left hand axis, and the integrated density values for RII and the reactivity of RII fragment (Rfr) on the right hand axis. Panel B shows parotid α-amylase Western blotting. The ordinate shows integrated density, and the error bars show ± 5% error. F, flight; C1, vivarium control; C2, habitat control.
Figure 7. Microarray analyses of selected parotid genes. The ordinate shows the log2 values of the ratio of the means of the normalized signal intensities determined for each gene for flight vs. habitat control. Prkar2a, protein kinase, cAMP dependent regulatory, type II alpha; Akap13, A-kinase anchor protein 13; Pde4a, phosphodiesterase 4a, cAMP specific; Adrb2, adrenergic receptor, beta 2; Adcy3, adenylate cyclase 3; Amy1, amylase 1, salivary.

Table 3. Ratios of RII to Total Protein of STS-135 and Bion-M1 PAGE.

<table>
<thead>
<tr>
<th></th>
<th>STS-135</th>
<th></th>
<th>Bion-M1</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PAGE</td>
<td>F</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>STS-135</td>
<td>70.8</td>
<td>69.28</td>
<td>69.99</td>
<td></td>
</tr>
<tr>
<td>RII</td>
<td>5.16</td>
<td>41.43</td>
<td>24.24</td>
<td></td>
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<tr>
<td>Ratio</td>
<td>0.073</td>
<td>0.60</td>
<td>0.35</td>
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</tr>
<tr>
<td>Bion-M1</td>
<td>153.6</td>
<td>129.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>RII</td>
<td>21.7</td>
<td>16.26</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>0.14</td>
<td>0.13</td>
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</tbody>
</table>

Values are integrated density measurement for total protein and RII Western blots when flight (F) samples are compared to those of vivarium controls (C1) and habitat controls (C2).
Table 4. Microarray Analyses of Secretory Protein Gene Expression.

<table>
<thead>
<tr>
<th>Gene</th>
<th>STS-135 Log2 F/H</th>
<th>STS-135 Fold Change F/H</th>
<th>Bion-M1 Log2 F/H</th>
<th>Bion-M1 Fold Change F/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy1</td>
<td>0.030</td>
<td>1.02</td>
<td>0.169*</td>
<td>1.124</td>
</tr>
<tr>
<td>Bpifa2</td>
<td>0.241§</td>
<td>1.18</td>
<td>0.170*</td>
<td>1.13</td>
</tr>
<tr>
<td>Lpo</td>
<td>0.070</td>
<td>1.05</td>
<td>0.160*</td>
<td>1.18</td>
</tr>
<tr>
<td>Lyz1</td>
<td>0.317</td>
<td>1.25</td>
<td>0.898∞</td>
<td>1.86</td>
</tr>
<tr>
<td>Pip</td>
<td>0.228*</td>
<td>1.17</td>
<td>0.169*</td>
<td>1.13</td>
</tr>
<tr>
<td>Prpmp5</td>
<td>-0.161§</td>
<td>0.894</td>
<td>-0.861∞</td>
<td>0.550</td>
</tr>
</tbody>
</table>

* Amy1, amylase 1, salivary; Bpifa2, BPI fold containing family A, member 2 (parotid secretory protein); Lpo, lactoperoxidase; Lyz1, lysozyme 1; Pip, prolactin induced protein; Prpmp5, proline-rich protein MP5

DISCUSSION

Space travel is ideal for studying the effects of an environment that cannot be effectively reproduced on Earth. The animal samples obtained from the space missions are unique and the experiments have to be precise and unambiguous. For that reason, we have selected three different experimental approaches (electron microscope based, biochemical, and molecular) to ensure the experimental results are verified by using several methods and the likelihood of technical variability is significantly reduced. The expression of secretory proteins is affected by a variety of physiological, pathological and, as is the case in microgravity, environmental factors. Secretory proteins themselves are important indices of the various functions of an organism. Proteins secreted into saliva serve as markers of the various cell types of the gland and the condition of the oral cavity and the proximal portion of the alimentary tract. In addition, secretory proteins representing other physiologic functions are present in saliva and can serve as
markers for monitoring the stability of those functions.

The proteins selected for study in these experiments are representative of digestive functions [α-amylase (Valdez and Fox, 1991); calcium and polyphenol binding [PRPs (Bennick, 1982; Bennick, 2002)]; antimicrobial [PSP (Geetha et al., 2003) and PRPs (Robinovitch et al., 2001)]; microbial binding activity [α-amylase (Scannapieco et al., 1993), PSP (Robinson et al., 1997), and DCPP (Ambatipudi et al., 2010)]; and regulatory activity [PKA RII (Daniel et al., 1998; Gold et al., 2013)]. In the parotid gland, α-amylase, PRPs, and PSP are products of acinar cells; DCPP is secreted by intercalated duct cells; and PKA RII is present in acinar (Mednieks et al., 1987) and striated duct cells (Piludu et al., 2002), and secreted by acinar cells (Mednieks and Hand, 1984).

It is possible differences in diet may have an effect on salivary glands. Parotid gland atrophy, gland weight, and enzyme activity decrease in rats fed liquid diet (Hall and Schneyer, 1964; Johnson, 1982; Zelles et al., 1989; Takahashi et al., 2012). Additionally, acinar cells exhibit increased numbers of autophagic vacuoles and increased apoptosis (Hand and Ho, 1981; Takahashi et al., 2012). However, in both STS-135 and Bion-M1 flight mice there were significant differences in protein expression, as well as an increase in autophagy and apoptosis compared to their respective habitat control glands, suggesting that regardless of diet, these processes were affected by microgravity. The presence of secretory granules and secretory proteins in autophagic vacuoles suggests granule exocytosis may have decreased, with prolonged retention of granules within the cytoplasm. Moreover, the increase in amylase protein shown by immunogold labeling in the Bion-M1 flight mice is opposite than expected for liquid diet (Zelles et al., 1989). Interestingly, liquid diet feeding has no apparent effect on rat submandibular and sublingual glands (Takahashi et al., 2014).

Vacuoles containing acinar secretory proteins in the apical cytoplasm of intercalated and striated duct cells of flight mice indicate endocytosis of proteins from the lumen. Whether this is a result of altered duct cell function or altered protein structure is unknown. Similar acinar protein containing vacuoles were seen in diabetic rats (Lotti and Hand, 1988), and duct cells avidly endocytose foreign proteins introduced retrogradely into the duct system (Hand et al., 1987).

The results from Western blotting are consistent with those of immunocytochemistry and microarray analyses. As reported previously (Mednieks et al., 2014), an important change occurs in parotid RII during short-term flights, where secretory protein expression is initially affected by microgravity. In the case of RII, it appears to return to basal levels during the longer Bion-M1 flight. This apparent stabilization may be a homeostatic response, at least in the catecholamine regulated secretory pathway. The mechanism for the stabilization might be related to the decrease in PDE and an increase in the expression of the adrenergic receptor, thus pushing the reaction to counter a possible decrease of RII expression. The submandibular gland, however, shows a much smaller component of RII and it apparently is unaffected (data not shown) by microgravity conditions. Parotid α-amylase initially is decreased but during the longer flight returns to baseline and, subsequently, higher levels than controls.

The microarray analyses confirm microgravity affects the expression of signaling pathway and secretory protein genes in salivary glands. The effects on expression of some genes were similar in both flights (e.g., Prkar2a and Pde4a were both decreased), although Prkar2a showed a return to baseline in the longer flight, while Pde4a was further decreased. The flight duration affected some genes in an opposite manner (e.g., the expression of Akap13 was increased in the shorter flight but decreased in the longer flight), whereas the expression pattern of Adrb2 was the reverse.

Although the microarray results revealed significant changes in the expression of several secretory protein genes, only two, Lyz1 and Prpmp5, showed a substantial fold change in expression with the longer Bion-M1 flight. Interestingly, these changes were in opposite directions — the expression of Lyz1 being increased and Prpmp5 being decreased. The changes observed by the three analytical approaches were not always coincident or of corresponding magnitude. For example, immunogold labeling showed a significant
increase in PRP expression on STS-135 and a small decrease on Bion-M1, whereas the microarray results indicated a small decrease in Prmp5 expression on STS-135 and a large decrease on Bion-M1. Similarly, α-amylase showed an almost 50% increase by immunogold labeling of Bion-M1 samples, but only a 12% increase in Amy1 expression by microarray and an apparent decrease by Western blotting. It is apparent that secretory protein expression under microgravity conditions is individually affected, rather than an overall response. Whether these differences result from different rates of transcription and translation, or different rates of protein and mRNA turnover, is unknown, and further investigation is required for a better understanding.

Other differences between the STS-135 and Bion-M1 missions included the age and sex of the mice. Both male and female mice are considered sexually mature at about eight weeks of age (Fox et al., 2007). The parotid gland reaches its definitive morphology by about four weeks of age (Ribeiro Castro et al., 2006). The expression of secretory proteins during mouse parotid gland development has not been thoroughly documented; however, in the rat, amylase, DNase, and RNase activities are essentially at adult levels by 40 days of age (Redman and Sreebny, 1971). Few, if any, differences have been described between male and female parotid glands; therefore, the age and sex differences of the mice on the two flights are not expected to have effects on secretory protein expression. There are significant morphologic, biochemical, and functional differences between male and female submandibular glands (Pinkstaff, 1998), but in this report we present only the results from the Bion-M1 mission.

Although the effects of different modes of post-flight transport are not known, recent findings indicate the difference in tissue harvesting times between the STS-135 and Bion-M1 missions were unlikely to have had a significant effect on our results. Studies of microvascular vasoconstrictor responses of vessels from mice allowed to recover for one day after landing (STS-133) were qualitatively similar to those of vessels obtained from mice within two to five hours after landing (STS-131) (Behnke et al., 2013). At five and seven days after landing, vasoconstrictor responses were similar to those of vessels of ground control mice. Preliminary data (unpublished) on the expression of selected parotid proteins from STS-133 mice show values similar to those of STS-131 mice, and a recovery to ground control values by five days after landing.

Thus, the major effects on changes in secretory protein expression observed in the present study are likely to be due to microgravity. Early hypergravity (1.7xg) and adrenergic stimulation experiments showed no short duration effect (Mednieks et al., 1998). Catecholamine analog (isoproterenol injections) stimulation showed a short and transient initial response, as might occur during lift-off or re-entry, but extended stimulation showed a more lasting and measurable effect (Mednieks and Hand, 1984).

As the salivary glands are an advantageous experimental system of the mouse model, so their secretory proteins are convenient for study and eventually for application to measuring human responses (Mednieks et al., 1994). In this study three approaches — immunocytochemical, biochemical, and molecular — were employed to determine the effects of microgravity. The findings of these experiments were consistent in the changes that were observed. The results indicated the effects may be time-dependent. Namely, the shorter flight showed an effect on the major salivary proteins that appeared to be diminished after the longer Bion-M1 mission. The longer exposure to microgravity may result in a homeostatic response (e.g., Selye, 1973).

**SUMMARY AND FUTURE GOALS**

Travel in space has an effect on oral tissues and specific secretory proteins. Tissue changes appear to involve autophagic, apoptotic, and endocytic activity. Molecular changes — especially those associated with catecholamine hormone responses — are affected, but tend to return to basal levels on longer flights.

Future goals are to elucidate specific metabolic pathways involved in altered physiologic functions and to use secretory proteins as markers for these events. In progress is the design of an economical, practical testing device for measuring markers in a bio-fluid for
use in space travel and adapted for clinical use on Earth.

AKNOWLEDGEMENTS

Our thanks and appreciation of the expert technical assistance of Ms. Maya Yankova, and for the use of the instruments of the Central Electron Microscope Facility at the University of Connecticut Health Center. Participating students included Renee Rubenstein, Christopher Haxhi, Tabrez Adil, and Ly Dang. We also thank Drs. W.D. Ball, D.M. Carlson, and L. Mirels for providing antibodies for this study. Supported by NASA grant NNX09AP13G (MIM); CT Space Grant College Consortium (ARH); Roosa Family Foundation Memorial Fund of the Hartford Foundation for Public Giving; School of Dental Medicine (SDM) Alumni Research Funds; and the University of Connecticut Health Center (UCHC) High School Student Research Apprentice Program.

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The Effects of the Spaceflight Environment on the Vaginal Mucin Layer of the Mouse

Camille Romer and Allan Forsman

Department of Health Sciences, East Tennessee State University, Johnson City, TN 37614

ABSTRACT

It has been well documented that spaceflight has adverse effects on many tissues and systems throughout the body. Although this phenomenon is well documented, relatively little research has been done in the area of the female reproductive system. If spaceflight has harmful effects on the female reproductive system, the migration of the human species into space would be greatly compromised. The purpose of this study was to determine the effects of spaceflight on the thickness of the apical mucin layer in the vaginae of mice, as changes in this layer could have detrimental effects on sperm survival and, therefore, a profound impact on the animal’s ability to reproduce. This study examined the thickness of the vaginal mucin lining from female mice that were exposed to 13 days of spaceflight and their concomitant controls. The tissues were stained using a technique commonly used to localize and analyze mucin varieties. The tissue was qualitatively analyzed for the type of mucin produced (i.e., acidic, neutral, acidic/neutral mixture). Further, the tissue was quantitatively analyzed for the amount of mucins produced by measuring the thickness of the mucin layer. The results of this study indicate that spaceflight causes a thickening of the mucin lining of the vaginal canal. The results further indicate being housed in an Animal Enclosure Module also caused a thickening of the vaginal mucin layer—presumably due to internal cage environmental factors—but this effect was not as pronounced as that seen in the spaceflight mice.

INTRODUCTION

Many studies have documented the effects of spaceflight and simulated microgravity on various tissues and systems of the body. Some of these systems include, but are not limited to: skeletal muscle arterioles and regional blood flow (Arbeille et al., 1996; Delp, 1999), the skeletal system (Droppert, 1990; Ferguson et al., 2002; Milstead et al., 2004), the immune system (Armstrong et al., 1993; Chapes et al., 1993; Chapes et al., 1999; Sonnenfeld et al., 2003), the anterior pituitary (Pattison et al., 1991), and the seminiferous tubules of the male reproductive system (Kamiya et al., 2003; Motabagani, 2007; Forsman, 2012). Overall body fluid shifts have also been reported (Tipton et al., 1987). Unfortunately, investigations into the effects of spaceflight on the reproductive system—especially the female reproductive system—have been limited. If the reproductive system is adversely affected by spaceflight, human colonization of space would be problematic for our species.

Key words: Microgravity; Spaceflight; Mucin; Vagina; Mouse

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Due to the lack of research in this area, the 2011 Decadal Study on Biological and Physical Sciences in Space (National Research Council Decadal Survey, 2011) developed two overarching questions regarding the reproductive systems. The first overarching question of the Decadal Study regarding the reproductive systems was to determine whether successive life cycles could be completed in a microgravity environment. The second question was to determine if reproduction would be affected in spaceflight.

Reproduction — especially in regard to the female — is a multifaceted process. To fully investigate the questions brought forth in the Decadal Study, one must consider several linked factors. First, the production and ovulation of a viable secondary oocyte must be determined because reproduction cannot occur without a viable secondary oocyte. Second, the structures of the female reproductive system that will be responsible for the care and transport of the oocyte (as well as potential zygote/embryo), and subsequent implantation and development to term, must be investigated. This requires studies of the uterine (fallopian) tubes and the uterus. If the reproductive process proceeds to term, will the process of labor be affected by microgravity?

Additionally, it is imperative the female reproductive structures are studied in regard to their ability to interact with the male and, subsequently, successfully care for and transport spermatozoa to the secondary oocyte. This requires not only the study of the previously mentioned female structures, but also studies involving the vagina, as the vagina is responsible for receiving the sperm. If vagina/sperm interaction is modified, it could affect the ability of the sperm to travel farther into the female reproductive system. If sperm are not able to reach the secondary oocyte, there will be no fertilization and thus, no reproduction. Only a few of these factors have previously been addressed.

Mucins are glycoproteins that contain large numbers of O-linked oligosaccharides. Mucins are believed to provide several functions, such as lubrication and protection from pathogens. If one considers the fact that increased microbial virulence has been documented in spaceflight conditions (Klaus and Howard, 2006; Rosenzweig et al., 2010), the fact that mucins play a role in protection from pathogens becomes increasingly important.

Mucins are found on the apical surface of many epithelia — including the uterine tissue (Gipson et al., 1995) — and are essential to reproduction in the female. Mucin genes are widely expressed in the tissue of the reproductive tract and each reproductive organ expresses a particular set of these genes (Lagow et al., 1999). It has been shown the expression of these genes can be influenced by steroid hormones and various disease states (Hebbar et al., 2005). This entices the question: “Can gene expression be altered by the spaceflight environment?” The mucins that line the female reproductive tract provide an environment conducive to sperm maturation, gamete interaction, and early embryonic development (Gandolfi et al., 1989). Eight varieties of mucins have been related to the female reproductive tract in humans. These have been designated as MUC1-MUC7, with two subsets of mucins. All epithelia of the female reproductive tract express MUC1, which is a transmembrane mucin produced by most epithelia (Warren and Spicer, 1961). Mucins that contain sialic acid are commonly referred to as sialomucin complexes (SMC). MUC4/SMC is expressed at the apical surface of most epithelia of the female reproductive tract, including both uterine luminal and glandular epithelium. These SMCs have been shown to block cell and molecular recognition processes, which renders the apical surface of cells with this type of mucin non-adhesive (Carraway et al., 1992). This type of mucin has been found to be hormonally regulated in uterine luminal epithelium, but not in uterine glandular epithelium, vaginal epithelium, cervical epithelium, or the epithelium of the uterine tube (Idris and Carraway, 1999). McNeer et al. (1998) and Carraway and Idris (2001) reported SMC expression is tightly regulated in the uterus and its expression appears to block blastocyst implantation.

Studies of the apical mucin layer that lines the various regions of the female reproductive tract in mice indicate microgravity has varying effects on the mucin layer thickness, depending on the region involved. This is not unexpected since the mucin lining in each region of the female reproductive tract has a different function. Examination of the uterine tissue from STS-118
indicated spaceflight caused a thickening of the apical mucin layer (Forsman and Nier, 2013). This is a potentially significant finding since thickening of this layer would provide a greater barrier to blastocyst implantation (Carraway and Idris, 2001), resulting in a decreased chance of pregnancy.

Examination of the apical mucin layer of the uterine tubes from the same mice indicated the effect of spaceflight varied depending on the region of the uterine tube. Svalina and Forsman (2013) found in all regions of the uterine tube the baseline animals always had the thickest mucin layer. The mucin layer of the ampulla region from spaceflight animals was significantly thinner than that of the baseline animals. No significant change was found in the mucin layer of the infundibular region of the tube, regardless of treatment type. This is a potentially significant finding since the uterine tube functions for the transport of gametes and is an active secretory organ whose secretions provide a suitable environment for continued maturation of male gametes, interaction between gametes, and early embryonic development (Gandolfi et al., 1989). Modification of the mucin layer in the uterine tubes could have detrimental consequences on the survival of the oocyte, sperm, or embryo. If one or any combination of these three is impaired, the chances of a successful pregnancy are greatly reduced.

Mucins of the vaginal canal provide lubrication and protection from microbial invasion and infection (Idris and Carraway, 1999). The general acidity of vaginal mucin is the female reproductive system’s first line of defense against possible microbial invasion. Normal vaginal pH is between 3.5-4.0 (Masters and Johnson, 1966). This pH range is generally inhospitable to many harmful bacteria and, therefore, is necessary to provide the female reproductive tract with some defense against possible invading harmful microorganisms. These vaginal and cervical mucins also play a role in sperm capacitation/motility. The optimal pH for sperm motility is between 7.0 and 8.5 (Tampion and Gobbons, 1963; Moghissi et al., 1964), and sperm motility has been shown to be reduced at pH values lower than 6.0 (Markler et al., 1981; Peek and Matthews, 1986). It has been shown that vaginal pH rises to approximately 7.0 within seconds after the introduction of ejaculate (Fox et al., 1973). If vaginal mucins were to become more acidic than their normal state, it may result in vaginal pH remaining too low (below pH 7.0) and render an environment inhospitable to sperm, resulting in reduced fertilization (Brannigan and Lipshultz, 2008). With this in mind, this research project focused on changes in the female reproductive system by examining the mucin layer of the vaginal tissue of spaceflight mice.

MATERIALS AND METHODS

Twelve (12) C56BL/6 female mice (Charles River, Wilmington, MA) were flown on NASA space shuttle mission STS-118 in August 2007. The mice were approximately eight weeks old at the time of launch. The duration of the spaceflight subjected these animals to approximately 13 days of microgravity. The flight (FL) mice were housed in the animal enclosure module (AEM) of the Commercial Biomedical Testing Module-2 (CBTM-2), which was part of the payload in the shuttle’s mid-deck flight locker. A set of 12 analogous mice — considered ground control (GC) mice — were housed in similar ground-based AEM/CBTM-2 enclosures. These ground-based enclosures and GC mice were run at a 48 hour delay in relation to the FL mice. This allowed for reproducing the same environmental factors as those experienced onboard the space shuttle (i.e., temperature, humidity, and light/dark cycles), except for the microgravity environment. A third set of 12 mice, considered baseline (BL) mice, were kept in standard rodent cages in standard conditions: 12/12 light-dark cycles at room temperature. Both BL and GC samples were maintained in the Space Life Sciences Lab, Kennedy Space Center, FL. Within a few hours of mission completion, the FL mice were transferred from the shuttle mid-deck flight locker to the Space Life Sciences Lab where, within minutes, the vaginal tissue was harvested from each mouse and the preservation steps initiated. Preservation involved immersion of tissues in 4% paraformaldehyde in 0.1 M PO₄ buffer (pH 7.4) for 12 hours at 4°C. This was followed with three washes in 0.1 M PO₄ with 2% sucrose and 50 mM NH₄Cl (pH 7.4) for 1 hour at 4°C and two washes in 0.1 M PO₄ with 50 mM NH₄Cl (pH 7.4) for 1 hour at 4°C. The tissues were then dehydrated using increasing concentrations of...
ETOH and embedded in paraffin using standard embedding techniques. The tissue was stored until use in this study.

Each sample was sectioned at four microns using a Microm HM325 microtome, mounted on glass slides, and stained using an Alcian Blue Periodic Schiff procedure. The tissue was then dehydrated, cover-slipped, examined, and photographed using a Zeiss Axioskop 40 microscope equipped with a Canon Powershot A640 camera. For each animal sample, three slides were prepared. Measurements were made using the Carl Zeiss AxioVision software, version 4.7.0. Using a randomization grid, a set of five random measurements of the thickness of the mucin layer was made from each of the three slides, giving a total of 15 measurements per sample. The average mucin thickness (in micrometers) was then calculated for each sample. A one-way ANOVA was made using MiniTab statistical software. The stained tissue was also qualitatively analyzed for the type of mucin present (i.e., acidic, neutral, a mixture of acidic and neutral) based on the color of the stained mucin. Using this staining technique, neutral pH mucins stain magenta, acidic mucins stain pale blue, and mixtures of acidic and neutral mucins stain purple. The results are based on seven flight samples, 12 ground control samples, and 10 baseline samples.

RESULTS/DISCUSSION
Because this is a study of the female reproductive system, one must consider the stage of the estrous cycle between animals when making comparisons. Due to the tissue sharing nature of these animals, it was not possible to obtain blood hormone data or vaginal smears to indicate the stage of estrous of each individual animal. However, due to the numbers of female mice per cage and the absence of any male mouse or male mouse excreta, it is reasonable to assume that the Lee-Boot effect (Whitten, 1959) had synchronized all of these animals into an extended period of diestrus. Thus, hormonal changes due to estrus were eliminated and not considered as variables. The thickness and general pH of the apical mucin layer of the vaginal canals from BL mice (Figure 1), GC mice (Figure 2), and FL mice (Figure 3) were quantitatively and qualitatively compared.

Figure 1. Vaginal epithelium from Baseline tissue (400X). Mean mucin layer thickness for Baseline tissue = 6.5633 µm. Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).
Figure 2. Ground Control Tissue (400X). Mean mucin layer thickness for Ground Control tissue = 10.0310 µm. Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).

Figure 3. Flight tissue (400X). Mean mucin layer thickness for Flight tissue = 17.3520 µm. Note the pale blue staining of the mucin layer indicative of an acidic mucin (arrow).
Our studies indicate the mucin layer of the GC tissue was thicker than that of the BL, but this thickening was not statistically significant (P=0.522). However, the results showed the thickness of the mucin layer of the BL compared to the FL mice was significantly different (P=0.040). Interestingly, the mucin thickness of the FL mice was not significantly thicker than that of the GC mice (P=0.211) (Figure 4). The mucin layer of the FL mice was visually much thicker compared to the other two groups. For seven of the BL tissues and six of the GC tissues, the mucin layer was too thin to be measured. It is important to note this was never the case for any of the FL tissues. The mucin layer for all samples was qualitatively examined to determine the general pH of the mucin. Neutral pH mucins stain magenta, acidic mucins stain pale blue, and mixtures of acidic and neutral mucins stain purple. All tissue samples in this study were found to be acidic in composition, regardless of treatment group.

These studies show there was a trend toward a thicker mucin layer of the vagina between the BL and GC mice. This is very interesting because it implies the AEM itself had some effect on the mucin layer — regardless of the gravitational situation — but that spaceflight also added to the thickening of the mucin layer. A possible explanation for this observation is mice are generally social animals that normally burrow into their bedding and sleep huddled with other mice. The AEM does not contain bedding; therefore, the mice (both GC and FL) are unable to bury themselves in bedding, possibly affecting the animals’ ability to maintain a normal body temperature. This could also explain the difference between the GC and FL mice, because the GC mice could still huddle and sleep together for warmth, whereas the FL mice — due to the nature of the microgravity environment — would have difficulty huddling while awake and would not be able to huddle while sleeping. This could make it even more difficult for the mice to adequately maintain normal body temperature, and consequently put these animals in a distressed state. There are also other environmental factors of the AEM that do not exist with standard rodent

![Mean Vaginal Mucin Layer Thickness](image)

**Figure 4.** Graph comparing the mean apical mucin thickness between the BL, GC, and FL animals

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cages, such as noise from the AEM ventilation system and a constant air flow, both of which could also present a stressful situation for the animals. Previous studies by Castagliuolo et al. (1996) indicated 30 minutes of immobilization stress can cause a significant increase in colonic mucin release in rats. This is a good indicator there is a need for further research to investigate the possible effects of stress on mucin release in the various regions of the female reproductive system.

The factor of spaceflight responsible for causing a thickening of the vaginal mucin layer is yet to be determined. It is possible the thickening is due to the microgravity of spaceflight, but it could also be caused by the high dosage of cosmic radiation experienced by the spaceflight mice. Experiments are currently being conducted to test this hypothesis. As previously stated, it has been well documented spaceflight has adverse effects on the immune system. One of the functions of vaginal mucin is to act as a barrier to possible invading microbes. Vaginal mucin is normally acidic in nature, which retards bacterial reproduction. Perhaps the thickening of the vaginal mucin in spaceflight is a response to ensure the animal will not contract an infection through the vaginal canal. Such an infection would be hard to fight for an animal with a depleted immune system. This thickening of the acidic vaginal mucin layer could also interfere with the ability of the female mouse to become pregnant, since acidic environments are highly spermicidal.

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Prolonged Head-Down Posture of Bats Induces Remodeling of the Aorta

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ABSTRACT

Inversion is the regular position for bats at rest, but continuous inversion was expected to reverse the gravity vector exposure from feetward to headward and present hemodynamic challenges that induce remodeling of the aorta. There is paucity of information regarding the cardiovascular structural adaptations in bats engaged in regulating cranial or caudal blood redistribution in prolonged inversion. The aim of this study was to determine aortic adaptations in bats during prolonged inversion. Forty (40) bats were captured at Iwo, Osun State, Nigeria and randomly allocated into a normal control group and three test groups (n=10/group). The inversion period was not extended in control group A, but was maintained 8 days in B, 15 days in C, and 22 days in D. At the end of each inversion period, the bats were euthanized using intramuscular injection, and tissues were processed for Haematoxylin and Eosin, Orcein, and Van Gieson staining. Histological changes in the tunica media and adventitia were quantified, and the results were analyzed statistically. The ascending aorta exhibited thickening of the media and adventitia, whereas the abdominal aorta showed thinning of these regions. The changes increased in magnitude with longer periods of inversion. The histological stains indicated alterations in smooth muscle cells, collagen, and elastin content, consistent with predicted elevated pressure in the ascending and decreased pressure in the abdominal aortae. The vascular adaptation in bats may provide insights into suspected cardiovascular changes in astronauts during long-term spaceflight.

INTRODUCTION

In response to chronic head-down positioning, laboratory rodents develop increased intracranial pressure and reduced caudal pressure (Papadopoulos and Delp, 2002). Hindlimb unloading rodent models have been used in ground-based studies to simulate the mechanical alterations that occur with exposure to the microgravity environment of spaceflight (Colleran et al., 2000; Papadopoulos and Delp, 2002; White, 2005). In order to advance the understanding of the mechanisms of arterial adaptation, there are advantages to studying animals with unique challenges imposed by long periods of head-down tilt and extreme inversion, as occurs in bats.

The degree of inversion maintained by bats at rest is greater than that achieved in hindlimb unloaded rodents. Bats normally rest and sleep inverted for about 12 hours each day. Other times, they fly long distances and feed on fruit in non-
head-down postures. The specific objective of this work was to characterize the histological adaptation of the bat ascending and abdominal arterial walls during extended head-down posture.

MATERIALS AND METHODS

Acquisition of Animals

Forty (40) presumably healthy fruit bats (Eidolon helvum) (~300 g) were obtained in November-December 2011 by netting from the Bowen University Campus bat roosting colony, Iwo, Osun State, Nigeria. In this season, the bats performed routine daily flight and resting behaviors (i.e., they were not hibernating). Experimental procedures were approved by the Bowen University Bat Conservation Committees and Departmental Ethical Clearance Committee. All experimental procedures adhered to the legal requirements of animal research in Nigeria. Only male bats in good health were utilized for study. Female bats were returned to the colony. Captured bats were kept in wooden cages at controlled room temperature of about 26°C and photo-periodicity of 12 hours light and 12 hours darkness.

Experimental Design

The bats were divided randomly into four groups. Group A bats were taken directly into the laboratory for immediate euthanasia. Groups B, C, and D underwent inversion for 8, 15, and 22 days, respectively. The bats were singly-housed in wooden cages. Within the cage, the bats hung inverted by their hook-shaped hindlimbs that gripped the wire gauze roof of the cage. This is the normal position during sleeping and resting. Throughout the period of the experiment, the roof of the cages to which all the bats hung their hindlimbs was unopened. The bats hanging was also unrestricted. Cage design did not permit bats crawling to the base of the cages. Also, food and water were positioned on a platform close to the roof. The bats aligned their bodies parallel to the Earth’s gravitational field, except when eating.

Observation of Body Positioning

Body position of the bats was recorded twice a day (8 a.m. and 8 p.m.). Before readings were taken, ten minutes of waiting was observed after reaching the laboratory area where the bats were kept to limit bat reaction to human entrance. The bats adapted to human presence. Bats were recorded as being inverted when their trunk and head were tilted downward, and they were recorded as being non-inverted when their trunk and head were tilted upward or horizontally. It was observed that the bats were inverted in 98.2% of total observation and were non-inverted in only 1.8% of all observations.

Animal Euthanasia

The bats were weighed to calculate the dose of sodium pentobarbital (40 mg/kg) which was used for the euthanasia. When deeply anesthetized, a longitudinal incision was made through the mid-thoracic and mid-abdominal walls to obtain the aortae of the bats.

Tissue Processing

The ascending and abdominal aortae were carefully dissected and excised for histological processing as described by Culling (1974). The ascending aorta was excised at 1 cm away from its origin from the heart, and the abdominal aorta was excised at 6-7 cm distal from the heart. The tissues were fixed in 10% formal saline. The fixed tissues were dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin wax at 58°C in plastic cassettes. Cross sections were cut at 3 µm and affixed on clean slides. The serial sections were stained with Haematoxylin and Eosin (H&E, general structure), Orcein (elastic fibers), and Van Gieson (collagen fibers). Slides were examined by light microscopy and photomicrographs were taken with a high-definition digital camera, Leica ICC50 (Leica Microsystems, England), mounted on a microscope, Leica DM 750 (Leica Microsystems, England).

ScopePhoto 3.0.11(2007) software was used for histomorphometry at a final magnification of times 100. Every sixth serial section was analyzed to avoid replicate counting of measurement. For each parameter, measurements were taken from at least five sections from each animal. Values were averaged to generate means ± standard error of mean (SEM) for each group and used for statistical analysis. Vascular wall thickness was measured at four points (0°, 90°, 180°, and 270°) following the method of Lee, 1987. Tunica media thickness was measured by a line drawn from the
luminal to the adventitial margins of the tunica media. Tunica adventitia was measured by a line drawn from the media-adventitia boundary of the vessel wall (Lee, 1987).

**Statistical Analysis**

Values were expressed as means ± SEM and compared between the test groups and the control group using one-way Analysis of Variance, Scheffe’s post hoc test, and Least Significant Differences (SPSS statistical software, version 20). Multiple comparisons were also performed between all groups. Statistical significance was defined at a value of P < 0.05.

**RESULTS**

**Histomorphometric Observation**

*Ascending aorta*

Compared to control (A), the thickness of the tunica media of the ascending aorta is significantly greater after prolonged head-down posture with percentages of 35.0% (B), 49.8% (C), and 100.7% (D) (Figure 1). The adventitial thickness is also higher after longer inversion in B (132.4%), C (159.5%), and D (365.8%) compared with control (A) (Figure 1). The ratio of adventitia to media thickness is larger (1.25) in group D than control (A) (0.51).

*Abdominal aorta*

The thicknesses of the media compared to control (A) are significantly smaller after inversion by 25.6% (B), 47.3% (C), and 49.4% (D) (Figure 2). The adventitial thicknesses also decreased in B (28.0%), C (25.6%), and D (29.3%), compared with control (A) (Figure 2). The ratio of adventitia to media thickness is larger (1.00) in group D than control (A) (0.72).

**Histological Findings**

*Ascending aorta*

The tunica media is thicker in B, C, and D, compared to control (A), and filled with smooth muscle cells (Figure 3). In control (A), collagen fibers appear as tightly interwoven strands throughout the width of the tunica media (Figure

![Figure 1. Changes in ascending aorta tunica media and tunica adventitia with prolonged head-down posture. Values are mean ± SEM, n=5 in each group. * indicate statistical difference of B, C, D compared to A (P<0.05)](image-url)
Figure 2. Changes in abdominal aorta tunica media and adventitia with prolonged inversion. * indicate statistical differences in B, C, D compared to A (P<0.05)

Figure 3. Representative photomicrographs showing bat ascending aortic cross section for the groups A, B, C, and D with H&E staining for smooth muscle. A (control) smooth muscle component appears compacted. B (8 day inversion) smooth muscle component appears tortuous and spaces. C (15 day inversion) smooth muscle component appears tortuous with spaces. D (22 day inversion) smooth muscle component appears tortuous and compacted. Thick arrows indicate tunica media smooth muscle, thin arrows point to smooth muscle nuclei, and asterisks marks lumen. Magnification = x400.
In groups B, C, and D, collagen fibers fill the full width of the tunica media. Elastic fibers are present across the media (Figure 5). In control (A), elastin fibers are closely packed, whereas in groups B, C, and D, they are less densely packed and appear more tortuous and interwoven (Figure 5).

Figure 4. Representative micrographs showing ascending aortic collagen deposition for the groups A, B, C, and D with Van Gieson staining. A (control) showed collagen fibrillar bundles with close packing. B (8 days inversion) collagen fibers are straight and widened media filled with collagen. C (15 day inversion) showed highly tortuous and interwoven collagen fiber; widened media filled with collagen fibers are seen. D (22 day inversion) showed highly tortuous, interwoven, and widened media filled with collagen fibers. Thick arrows point to collagen fibers. Bent arrows indicate fibrocyte or smooth muscle nuclei. Asterisk indicates vessel lumen. Four point star lies in the tunica adventitia. Magnification = x400.
Figure 5. Representative photomicrographs of Orcein stained elastin fiber in bat ascending aortic cross sections for the control (A) and test groups (B, C, D). Thick Arrows point to elastin fibers. Asterisk marks vessel lumen. In A, elastin fibers are closely packed bundles in the tunica media. In B, C, and D, elastin fibers are less densely packed and appear more tortuous and interconnected. Elastin fibers populate the whole width of the wider tunica media in B, C, and D. Magnification = x400.

Abdominal aorta

The reduced thickness of the tunica media and adventitia is evident in H&E sections from groups B, C, and D compared to control (A) (Figure 6). There appears to be less collagen in the adventitia of groups B, C, and D compared to the robust collagen density in control (A) (Figure 7). Elastin fibers are not different in control and test groups (staining not shown).

DISCUSSION

Our study is the first to examine aortic and morphological adaptation in bat prolonged inversion. The aortic adaptations are region-specific involving tunica media and adventitia. There is markedly increased thickness of the tunica media of the ascending aorta and thinning of the media of the abdominal aorta. The observed aortic wall adaptation in the current study might
Figure 6. Representative H&E stained sections of the abdominal aortae of the test and control bats. The tunica media (m) in A (control) is thicker than in the test groups. In plate B (8 days inversion), the smooth muscle layer are arranged in parallel straight rows. The tunica adventitia (a) is thin and less dense than control. In plates C (15 day inversion) and D (22 day inversion), the smooth muscle is aligned straight and appears less dense than control. Tunica adventitia component has a loose and dense matrix than control. The star symbol marks the lumen. Magnification = x100.
Figure 7. Representative cross sections of the abdominal aorta in control (A) and test groups (B, C, D) Van Gieson stained for collagen. In A (control), collagen is intensively stained in the adventitia. In B (8 day inversion), reduction in collagen is evident in the tunica adventitia. In C (15 day inversion), collagen reduction is evident in the adventitia. In D (22 day inversion), tunica adventitia light staining indicates fewer collagen. The star marks the lumen. Magnification = x100.

have been potentiated by a shift of intravascular fluid from the caudal part of the bats to the cranial part and, consequently, exposed the ascending aorta to greater mechanical stress while the abdominal aorta was less stressed. The increased media smooth muscle adaptation in the ascending aorta of bats might produce enhanced myogenic responses (Heather et al., 2006). Gao et al. (2009) examined morphological adaptations associated with hindlimb unloading in rats. They observed that 28 days of head-down tilt induced mild hypertrophic changes in the common carotid artery of the abdominal aorta. Our findings in inverted bats are similar, but more striking than in the rat model (Tuday et al., 2007). Meanwhile, this may be due to greater angle of tilt (vertical to gravity vector) at which bats are inverted compared to rodents. The observed growth of the
ascending aorta could lead to aortic regurgitation and could partly contribute to cardiac myocyte damage seen in the bat heart in prolonged inversion (Ashaolu and Ajao, 2014). The rapid hypertrophy of the ascending aorta wall may be an adaptive response to counteract increased cranial pressure. Vascular physiological assessment is required to check for predicted functional changes.

Judging from the histological sections, the collagen and elastin content within the ascending aorta increased in-step with the smooth muscle growth. This is important for maintaining strength and function of the arterial wall under presumed conditions of increased pressure. Increased collagen is consistent with that reported for the thoracic aorta in head-down tilted rodents (Tuday et al., 2009).

Opposite to the hypertrophic response in the ascending aorta, the abdominal aorta exhibits involution. It would be of interest to compare the physiological responses of ascending and abdominal regions of the aorta to increased luminal pressure. Reduced wall thickness may result in hyporesponsiveness of the aortic segment pressure. It has been demonstrated in inverted laboratory animals that decreased pressure existed within the abdominal aortic column (Heather et al., 2006), and most studies have established atrophic changes in mesenteric vessels (Heather et al., 2006). The present study has demonstrated a progressive reduction in the wall thickness and collagen content in the abdominal aorta as inversion days progressed. The decreased collagen component of the abdominal aorta implies reduced stiffness. Previously, Ashaolu (2009) hypothesized that bat abdominal vessels may serve as blood reservoir during inversion. The present observations reveal a dramatic adaptation of the aorta, and changes are region-specific. With respect to stress on captive bats, our findings reveal that the housing conditions can profoundly change vascular anatomy. At a more general level, the plasticity of the aorta unveiled in this research suggests that humans in microgravity with altered blood flow and pressure distributions from the Earth may experience vascular remodeling that puts them at risk for cardiovascular malfunction during return to gravity.

CONCLUSION

The head-down posture resulted in wall thickening of the ascending aorta and thinning of the descending portions of the aorta. The structural changes are considered to be dramatic and consistent with regions of higher and lower intra-arterial pressures. This study sheds light on the arterial adaptations that are associated with profound gravitational vector alterations in bats. Further studies will be required to discover what extent this adaptability of arteries exists in other species, including humans.

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CONFLICT OF INTEREST

None.

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Over-Expression of FT1 in Plum (Prunus domestica) Results in Phenotypes Compatible with Spaceflight: A Potential New Candidate Crop for Bioregenerative Life Support Systems

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ABSTRACT

Tree fruits (e.g., apples, plums, cherries) are appealing constituents of a crew menu for long-duration exploration missions (i.e., Mars), both in terms of their nutritive and menu diversity contributions. Although appealing, tree fruit species have long been precluded as candidate crops for use in plant-based bioregenerative life support system designs based on their large crown architecture, prolonged juvenile phase, and phenological constraints. Recent advances by researchers at the United States Department of Agriculture (USDA) have led to the development of plum (Prunus domestica) trees ectopically over-expressing the Flowering Locus T-1 (FT1) gene from Populus trichocarpa (poplar). The transformed plants exhibit atypical phenotypes that seemingly eliminate the aforementioned obstacles to spaceflight. Here we demonstrate the FT1 expression system (FasTrack) and the resultant dwarf growth habits, early flowering, and continuous fruit production. The potential contribution of P. domestica as a countermeasure to microgravity-induced bone loss is also discussed.

INTRODUCTION

Tree fruits have often been deliberated on as potential crops in bioregenerative life support systems for use on long-duration missions (Wheeler, 2003). A continuous supply of fresh fruit could provide unique nutritive contributions to the crew’s diet and offer enhanced menu diversity, an important consideration on long-duration missions (Bourland et al., 2000). Regardless of their nutritional and menu diversification benefits, tree fruits have been precluded as candidate crops based on architectural, juvenility, and phenological constraints associated with their normal growth and development. Tree fruits are large, take a long time to mature, and in the case of temperate species, require a cold dormancy period between fruiting cycles. The dormancy requirement also means food production from these crops is periodic, which presents a further barrier to use in...
bioregenerative life support systems. There is also concern with tree crops regarding their harvest index (i.e., the ratio of edible biomass to total biomass), as trees tend to dedicate significant resources to the development of structural tissue (wood) relative to reproductive (edible) tissue.

Prunus domestica (plum) fruits are nutrient dense and serve as a good dietary source of potassium and vitamin K (Donovan et al., 1998; Stacewicz-Sapuntzakis, 2013; Stacewicz-Sapuntzakis et al., 2001). In addition, and particularly relevant to spaceflight, plums also produce a phytochemical complement that has been strongly linked to the prevention of bone loss and disease prevention in both pre-clinical rodent and human models (Shen et al., 2012; Stacewicz-Sapuntzakis, 2013). The combined nutritional benefits and bone loss mitigation potential make plums even more appealing as a crew menu item, should it be possible to overcome the barriers to growing them in space. Recent advances by researchers at the United Stated Department of Agriculture (USDA -Agriculture Research Service [ARS], Kearneysville, WV) have seemingly eliminated these barriers (Srinivasan et al., 2012; Srinivasan et al., 2014), thereby opening the door to the inclusion of tree fruit crops in bioregenerative life support systems for human space exploration missions.

TECHNOLOGY BACKGROUND

The FasTrack crop breeding system presented in Srinivasan et al. (2012; 2014) and briefly summarized herein, takes advantage of the Flowering Locus T1 (FT1) gene, which is a key flowering regulator in many higher plants (Navarro et al., 2011; Turck et al., 2008). P. domestica was transformed with the FT1 gene from Populus trichocarpa (poplar; PtFT1) using a 35S promoter (35S::PtFT1). The resultant ectopic over-expression of PtFT1 in P. domestica disrupts apical dominance, allowing axillary or secondary buds to develop into branches resulting in phenotypes ranging from bushy to creeping or planar growth habits (Figure 1 A-C), architectural phenotypes that appear to be compatible with spaceflight plant growth systems (Figure 1 B).

Early Flowering

Perhaps less obvious than the architectural barriers, but equally limiting in terms of spaceflight applications, is the prolonged period of exclusively vegetative growth that occurs in the juvenile phase leading up to the first flowering and fruit set. For P. domestica, this juvenile phase lasts between three to seven years (Srinivasan et al., 2012), making it impossible to produce a crop under any reasonable spaceflight scenario. In a terrestrial setting this is necessary to physically support the fruit against gravity; these structural support requirements would be greatly diminished in microgravity.

In addition to early flowering, the PtFT1 plum plants are small and can be grown for extended periods in pots. Further, there appears to be no obligate requirement for dormancy, although the plants do retain moderate cold hardiness. The lack of a dormancy requirement, coupled with continuous flowering, allows for continuous fruit production; however, the plants can be shifted between primarily vegetative or reproductive development through changes in temperature, with lower temperatures favoring reproductive development and higher temperatures favoring vegetative growth.

FT1 PLUM AND SPACEFLIGHT: REMOVING THE BARRIERS

Modified Plant Architecture

The most obvious obstacle for growing trees in a spaceflight environment is their large size. Typical mature plum orchard trees can range in height from 3-4 m (Day et al., 2013), making them impractical for use in any foreseeable spaceflight or planetary exploration plant growth system. In order to be considered as a candidate crop, the entire architecture of the tree needs to be reduced to the point that the tree could be grown in the same systems used for such candidate crops as tomato and pepper. The over-expression of PtFT1 in P. domestica disrupts apical dominance, allowing axillary or secondary buds to develop into branches resulting in phenotypes ranging from bushy to creeping or planar growth habits (Figure 1 A-C), architectural phenotypes that appear to be compatible with spaceflight plant growth systems (Figure 1 B).
PtFT1 transformed plums this juvenility phase is significantly reduced – in many cases to less than 12 months – such that comparatively small plants can develop numerous flowers and set large numbers of fruit (Figure 2).

Although the FT-plum development phase is still a somewhat protracted timeframe for experiments on the International Space Station (ISS), it should be noted the majority of this development time could elapse on the ground as a lead up to a spaceflight experiment. The already accelerated flower development associated with the PtFT1 expression can be reduced even further using clonal propagation from cuttage (Figure 2). Mature tissue can be excised from the parent plant and rooted to generate a large number of clonal plants that will establish roots and flower in as little as eight weeks from the time of cutting (Figure 2). Further, early propagation and spaceflight storage scenario results suggest both rooted and non-rooted cuttings can be stored (4°C; low or no light) for long periods of time (weeks to months) and remain viable (T. Graham, unpublished results), making the FT-plums further amenable to spaceflight experiments.

Figure 1. Altered plant architecture in PtFT1 modified Prunus domestica. Panel (A) illustrates the bush and planar growth habit induced by PtFT1 expression, relative to the non-transformed control plant (center). Panel (B) compares the relative size/architecture of PtFT1 plum and standard sweet bell pepper (Capsicum annuum ‘California Wonder’), suggesting the FT1 plums are, in terms of architecture, compatible with spaceflight plant production systems. Panel (C) further illustrates the altered morphology as well as the trainability of branches, which are vine-like in many cases (right).
Figure 2. Early flowering in plums propagated through cuttage. Panel (A): Eight-week old cutting shows early flowering. Panel (B): 14-week old cutting showing planar growth habit and abundant flower production. Panel (C): Four-week old rooted cutting ready for potting. Panel (D): Fruit set on a 12-week old cutting. Note the multiple fruit per flower.
Continuous Fruit Production

The use of bioregenerative systems as a crew food supply – in whole or part – during extended duration missions would require a constant production of foodstuffs (Wheeler, 2000). This can be accomplished with staggered plantings, or by using indeterminate crop species capable of continual food production. Although fruit trees are perennial and capable of multiple crops, most are not indeterminate for the purposes of bioregenerative life support. Most tree fruit production is phenologically regulated – particularly those species that evolved in temperate climates (Childers et al., 1995) – implying that sometime during a mission the trees would need to enter a cold dormancy phase, during which time they would neither produce food nor contribute to other life support functions (i.e., air revitalization and water purification). Under the influence of *PtFT1* over-expression, *P. domestica* has no obligate requirement for dormancy. New floral buds can be initiated, develop, and mature in the absence of a chilling phase (Srinivasan et al., 2012) allowing for the continuous production of fruit; essentially the plant has become indeterminate (Figure 3). This said, the degree of floral or vegetative bud development is modulated, to some degree, through changes in ambient temperature. Higher temperatures (e.g., 29°C) promote or favor the development of vegetative buds, while lower temperatures (e.g., 21°C) promote floral buds (Srinivasan et al., 2012; Srinivasan et al., 2014).

ADDITIONAL CONSIDERATIONS RELEVANT TO SPACEFLIGHT

Harvest Index

Harvest index is a plant productivity metric used to describe the relative distribution of biomass between the edible and inedible components of a crop (Hay, 1995). It was originally developed primarily for cereal crops but has since been used for a wide range of crops. Tree crops – such as plum – are generally considered to have a low harvest index, at least in the short term, as the plant directs its resources to the development of vegetative and structural elements (i.e., wood). In the long term (i.e., 20–40 years), it can be argued the harvest index is actually quite high given the multiple harvests, and field studies with ultra-dwarf fruit trees have shown biomass partitioning to fruit can be quite high once the trees develop beyond their juvenility phase (Palmer, 1988). The overexpression of *PtFT1* in *P. domestica* circumvents this barrier to spaceflight. The resultant early flowering, fruit production, and vine-like or bushy growth habits combine to increase the edible (fruit) biomass per unit total biomass (Figure 4), even in the first few years of growth.

Plums as a Countermeasure to Bone Loss

Bone loss and its impact on the health of crewmembers has been identified by NASA scientists as one of the greatest challenges to interplanetary space exploration and long-duration stays on the ISS. For crew members of the Russian MIR and ISS, the decrease in bone mineral density can range from of 1.0-1.6% per month in the hip and lumbar spine (LeBlanc et al., 2000). In the space environment, microgravity, radiation exposure, and immunological changes can all contribute to bone loss, but the most pronounced effects result from abnormal loading of the skeleton.

A variety of interventions, including exercise or loading regimens (Baldwin et al., 1996; Yang et al., 2009), drug therapies (Bikle et al., 1994; Turner et al., 1998), and dietary modifications (Globus et al., 2009; Smith et al., 2005; Zwart et al., 2004), have been considered as countermeasures. The appeal of dietary interventions is they could provide a practical and safe component to an osteoprotective regimen through the incorporation of foods rich in specific nutrients or non-nutrient bioactive components (e.g., polyphenolic compounds) or dietary supplements. The ideal dietary intervention would have the capacity to suppress the catabolic activity of the osteoclast cells (i.e., resorption), while maintaining or up-regulating the anabolic activity of osteoblast cells (i.e., formation). Additionally, *in vivo* and *in vitro* findings indicate free radicals and pro-inflammatory cytokines generated in the space environment can have detrimental effects on bone (Garrett et al., 1990; Kondo et al., 2010). Thus, a dietary intervention with antioxidant activity to protect against oxidative damage could inhibit bone resorption and stimulate bone formation.
Figure 3. Continuous fruit production in *PtFT1* *P. domestica*. Panel (A) demonstrates the coexistence of flowers, immature, and mature fruit on a vine-like branch. Panel (B) is a further example of new flowers developing on the same branch that is supporting mature fruit. Panel (C) is a close-up view of a mature plum fruit next to newly emerged flowers.
Figure 4. Relative abundance of edible and inedible biomass in PtFT1 modified *P. domestica*. Panel (A) illustrates a bush phenotype with numerous mature and immature fruit and flowers. The vine-like growth habit and high fruit-to-leaf ratio of this particular example elevate the edible to inedible biomass ratio to levels comparable to some other herbaceous crops, such as pepper and tomato. Panel (B) is a further example of the high harvest index potential of the FT-plum line(s).
Accumulated scientific evidence has demonstrated the beneficial effects of dried plums (*P. domestica* L. ‘Improved French’) on bone health. Several studies have shown dried plum supplementation prevents and even reverses bone loss in animal models (Figure 5) (Deyhim et al., 2005; Franklin et al., 2006; Halloran et al., 2010; Rendina et al., 2013; Smith et al., 2014a; Smith et al., 2014b). Importantly, it has also been shown that plum’s capacity to restore bone was similar to that of intermittent parathyroid hormone (PTH), the only FDA-approved bone anabolic therapy (Bu et al., 2007). These and other studies provide a significant body of evidence that suggests supplementation with plum is unique in its osteoprotective effects on bone.

In terms of their bioactive components, plums are considered a nutrient dense fruit serving as a good dietary source of potassium and vitamin K, as well as a rich source of phenolic compounds (Donovan et al., 1998; Kayano et al., 2004; Stacewicz-Sapuntzakis et al., 2001). Dried plum is of particular interest because it has received the highest oxygen radical absorbance capacity (ORAC) ranking among the most commonly consumed fruits and vegetables (McBride, 1999). In one of our recent studies, we showed an extract of plum phenolic compounds accounted for \( \geq 90\% \) of the effects of plum on bone in an aging osteopenic animal model (B.J. Smith, unpublished results). While we recognize other components in plum (e.g., oligosaccharide) likely contribute to the benefits of plum on bone due to their ability to promote calcium uptake by cells (Weaver, 2005; Weaver et al., 2011), our findings indicate the specific phenolic compounds – seemingly unique in plum – are in large part responsible for the beneficial effects on bone.

**ADVANCING FT-PLUM TECHNICAL READINESS LEVEL**

The FT-plum phenotypes address all the major obstacles that have prevented inclusion of tree fruits for bioregenerative life support applications; however, FT-plums are still at a low technical readiness level (TRL). Many questions need to be answered before FT-plums can be accepted as a candidate space food crop. Early questions are centered on basic horticultural management and production under spaceflight conditions, including performance under super elevated CO\(_2\) levels typical in crew cabin environments. Further considerations include, but are not limited to, growth and performance under a 24 h photoperiod, propagation and growth in available spaceflight hardware, pollination considerations in space, and the safety issues regarding the consumption of the genetically modified material. Research aimed at increasing the TRL of these FT-plums by addressing these issues is currently underway.

**SUMMARY**

The resultant phenotypes associated with the ectopic expression of *PtFT1* in *P. domestica* appear to eliminate the barriers that have historically precluded the inclusion of tree fruits – such as plum – in the design of bioregenerative life support systems for extended space exploration missions. Although the primary barriers have been eliminated, much work remains to fully develop the FT-plums for spaceflight. Responses to basic spaceflight conditions and expected crop production scenarios must be elucidated before *P. domestica* becomes a fully accepted candidate crop. Basic horticultural management protocols appropriate for spaceflight are the early research focus. Once the basic horticultural practices are established and the most suitable lines are selected, spaceflight experiments can be pursued to validate *P. domestica* as a candidate crop. Concurrent research efforts will also focus on evaluating the potential of plums as a bone density loss countermeasure.

**ACKNOWLEDGEMENTS**

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Figure 5. MicroCT images of the lumbar vertebra of the spine subjected to finite element analyses. Mice were sham-operated (SHAM) or ovariectomized (OVX) and allowed to lose bone for two weeks prior to starting dietary treatments: control, low dried plum (OVX/LDP), or high dried plum (OVX/HDP) diet. The images show that both the LDP and HDP restored bone microstructure and bone strength in a dose-dependent manner (i.e., red regions represent stronger bone). Dietary supplementation with dried plum has been shown to reverse bone loss in aging and gonadal hormone deficient mice.
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Graham et al. — Reconsidering Tree Fruit for Bioregenerative Life Support

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Research Article

Comparative Toxicity of Lunar, Martian Dust Simulants, and Urban Dust in Human Skin Fibroblast Cells

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ABSTRACT

The National Aeronautics and Space Administration (NASA) has plans to further their manned space exploration to Mars and possibly beyond. The potential toxicity of lunar and Martian dusts to astronauts is a big concern. Primary routes of exposure for astronauts are dermal contact, ocular contact, and inhalation. In this study, we focused on dermal contact exposure using human skin cells to investigate the cytotoxic and genotoxic effects of two fractions of lunar dust simulant (JSC-1A-vf, JSC-1A-f) and a Mars dust simulant (Mars-1A), and compared them to urban dust (urban particulate matter), as urban dust toxicity is better understood and thus, provides a good comparison. Our data show the three simulants and urban dust are cytotoxic to human skin cells. The JSC-1A-vf lunar dust simulant is more cytotoxic than the JSC-1A-f and urban dust. Urban dust cytotoxicity is similar to Mars dust simulant after 120 h exposure. All three dust simulants and urban dust show similar low genotoxicity effects. Our data suggest extraterrestrial dust can damage skin cells and may have the potential to be harmful to humans.

INTRODUCTION

NASA has plans to further their manned space exploration to Mars and possibly beyond (Chatterjee et al., 2010). One big health challenge in these endeavors is the exposure to lunar and Martian dust. The toxicity of lunar dust is a major health concern for NASA, as it posed a significant health hazard to astronauts during the Apollo program and, thus, there is also concern about the toxicity of Martian dust. Lunar dust particles adhere to spacesuits and they enter astronaut living quarters in spacecraft, resulting in direct bodily contact (Wallace et al., 2009; Rehders et al., 2011; Wagner, 2006). During several Apollo missions, irritation of the eyes, respiratory system, and skin were reported, and it is believed these effects were a result of direct contact with lunar dust (Wagner, 2006).

The overall toxicity of extraterrestrial dusts (Martian, lunar, and other celestial bodies [e.g., asteroids]) is poorly understood. Martian dust is highly oxidized (Allen et al., 1998); however, its formation, composition, and physical properties

Key words: Lunar Dust; Mars Dust; Dust Simulant; Cytotoxicity; Genotoxicity; Skin Cells; Extraterrestrial Dust; Space Dust; Human Cells

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have not been fully characterized. Current data show Martian dust contains variable components, including known human carcinogens — such as chromium, nickel, and iron-rich silicate particles (Yen et al., 2005). Lunar dust is an ultra-fine, extremely abrasive substance with electromagnetic properties (Liu and Taylor, 2011) that cause the dust to stick to astronauts’ suits, boots, and equipment and makes them extremely difficult to clean. Lunar dust contains many different components, including chromium and silicates (McKay et al., 1994). The chemical and physical analysis of lunar and Martian dust suggests these dust particles could potentially contribute to the development of the disorders some of the astronauts presented during Apollo missions (Wagner, 2006).

There are only a few studies that have focused on the toxicity of extraterrestrial dust and their indicated toxic effects. One study reported lunar dust simulant causes a regeneration delay in cultured human keratinocyte monolayers (Rehders et al., 2011). The same study also showed Martian dust simulant induces cytotoxicity in human keratinocytes and CHO-K1 fibroblasts (Rehders et al., 2011). Latch et al. (2008) reported lunar and Martian dust simulants decrease the viability of human alveolar macrophages. Animals studies have shown that rats exposed to high doses of lunar dust via inhalation exhibit inflammation and lesions in the lung (Lam et al., 2013). Following intratracheally instilled exposure to lunar and Martian dust simulants, and then sampled at 7 or 90 days post exposure, mice lung tissues showed signs of inflammation and fibrosis at 7 days, but not 90 days (Lam et al., 2002a). It was also reported following acute exposure (24 h) to lunar and Martian dust simulants, mouse lung tissue showed signs of inflammation, while 4 h exposures had no effects (Lam et al., 2002b). Lunar dust was shown to cause minor ocular irritancy in vitro and in vivo (Meyers et al., 2012). In addition, lunar dust simulant showed neurotoxic potential by causing an increase in glutamate binding to the nerve terminal in rats (Krisanova et al., 2013). Based on various studies, the safe exposure levels of lunar dust for astronauts during long stays in habitats on the lunar surface is estimated to be 0.5-1 mg/m³ (James et al., 2013; Scully et al., 2013). It should be noted in addition to size and abrasiveness difference of lunar dust compared to simulant (e.g., JSC-1A-vf), lunar dust is considered more highly reactive (Liu et al., 2008; Park et al., 2008) and therefore studies using these simulants may underrepresent the toxicity of actual lunar dust in situ. Similar problems are present in regard to the Martian dust simulant — analysis of Mars dust shows some differences with composition and physical characteristics when compared to Mars-1A (Peters et al., 2008).

Despite the fact some of the species found in lunar dust are known human carcinogens (McKay et al., 1994), the genotoxicity and carcinogenicity of extraterrestrial dust have not been investigated. A primary route of extraterrestrial dust exposure is through skin contact; however, only one study considered the cytotoxicity in skin cells (Rehders et al., 2011). Accordingly, the objective of this study was to determine the cytotoxicity and genotoxicity of lunar and Martian dust in human skin fibroblasts and compare it to urban dust. Since urban dust toxicity is better understood, it thus provides a good toxicity comparison.

**MATERIALS AND METHODS**

**Cell Culture**

BJhTERT cells are hTERT-immortalized human skin fibroblasts. The cells exhibit a diploid karyotype and normal growth parameters. They were a generous gift from Jerry Shay of the University of Texas, Southwestern Medical Center. The cells were cultured in a 50:50 mixture of Dulbecco’s minimal essential medium and Ham’s F12 medium, plus 15% cosmic calf serum, 1% L-glutamine, 1% penicillin/streptomycin, and 0.1% sodium pyruvate. Cells were maintained in a 37°C, humidified incubator with 5% CO₂.

**Chemical Preparations**

We used extraterrestrial dust simulants developed by NASA from volcanic ash found in Arizona (for lunar) and Hawaii (for Martian) to facilitate testing of toxicity and system requirements for lunar exploration. We obtained two size fractions of lunar dust simulant, fine and very fine (JSC-1A-f and JSC-1A-vf, respectively), a Mars dust simulant (JSC Mars-1A, referred to as Mars-1A), and a representative urban dust (UPM) from St. Louis, Missouri. Stock solutions of the above compounds were prepared by suspending
them in a 50:50 mixture of Dulbecco’s minimal essential medium and Ham’s F12 medium. The stock solutions were sonicated prior to treatment. Cells were treated from the stock solutions at concentrations of 25, 50, 100, 200, or 400 µg dust/cm² dish surface area for 24 h or 120 h.

Cytotoxicity Assay

Cytotoxicity was determined using a clonogenic assay, which measures the reduction in plating efficiency of treatment groups compared to controls, as previously described (Wise et al., 2002). Each experiment was performed at least three times with four dishes per treatment group. Cells were treated directly with the intact dust particles (described above in “Chemical Preparations”) and a metal particle as a positive control. We found our positive control behaved as expected.

Clastogenicity Assay

Dust-induced clastogenicity was measured using the chromosome aberration assay, as previously described (Wise et al., 2002). One hundred metaphases per concentration were analyzed per experiment. Each experiment was repeated at least three times. Metaphases were analyzed for chromatid breaks, isochromatid breaks, chromatid exchanges, dicentrics, double minutes, acentric fragments, fragmented chromosomes, and centromere spreading. Cells were treated directly with the intact dust particles (described above in “Chemical Preparations”) and a metal particle as a positive control. We found our positive control behaved as expected.

Particle Size Characterization

Dynamic light scattering (phot on correlation spectroscopy) was used to measure the mass median diameter (MMD) of the four dusts in this study.

Statistics

Where mentioned, values are shown as mean ± SEM (standard error of the mean). Since the percentages calculated in repeated experiments at each treatment level are considered to be independent binomial measurements that can be approximated by a normal distribution, the standard independent two sample t test is valid to test the significance of differences between groups. It is expected that the variances of measurements at different treatment levels are different. We choose to use Satterthwart’s approximated t test, which assumes unequal variances between the two groups (p<0.05 was considered significant).

RESULTS

Particle Size Characterization of Dust Compounds

To determine the size of the dust compounds used, the mass median diameter of the four dust compounds was measured using dynamic light scattering (DLS). Table 1 reports the mass median diameters for JSC-1A-f, JSC-1A-vf, UPM, and Mars-1A; they are: 1.470, 0.726, 0.169, and 0.133 µm, respectively.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>MMD (µm)</th>
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<tbody>
<tr>
<td>JSC-1A-f</td>
<td>1.470</td>
</tr>
<tr>
<td>JSC-1A-vf</td>
<td>0.726</td>
</tr>
<tr>
<td>Urban dust</td>
<td>0.169</td>
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<tr>
<td>Mars-1A</td>
<td>0.133</td>
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Cytotoxicity of Dust Compounds

All compounds induced a concentration-related decrease in relative survival after 24 h exposure, plateauing in response after concentrations 100 µg/cm² (Figure 1). Mars-1A, JSC-1A-f, and UPM dusts induced similar cytotoxicity after 24 h exposure, while JSC-1A-vf was slightly less cytotoxic. For instance, concentrations of 25, 50, 100, 200, and 400 µg/cm² Mars-1A resulted in 100, 76, 68, 39, and 32% relative survival, respectively. The same concentrations induced 83, 87, 62, 67, and 62% relative survival for JSC-1A-f, respectively; 76, 62, 54, 44, and 26% relative survival for JSC-1A-vf, respectively; and 90, 69, 59, 52, and 42% relative survival for Urban dust, respectively.
relative survival for urban dust, respectively (Figure 1).

All the dust, except JSC-1A-f, also induced a concentration-dependent decrease in relative survival after 120 h exposure and a higher cytotoxicity compared to 24 h. Mars dust simulant had the highest toxicity. For example, concentrations of 25, 50, and 100 µg/cm² Mars-1A induced 54, 12, and 0% relative survival, respectively. JSC-1A-f at concentrations of 25, 50, 100, 200, and 400 µg/cm² induced 78, 78, 64, 60, and 51% relative survival, respectively. The same concentrations of JSC-1A-vf induced 78, 52, 24, 6, and 2% relative survival, respectively. Concentrations of 50, 100, and 200 µg/cm² UPM induced 52, 31, and 21% relative survival, respectively (Figure 2).

**Genotoxicity of Dust Compounds**

Next, we compared the genotoxicity of these four dusts. We used chromosomal aberrations as a measure of large-scale DNA damage. We only investigated the chromosome aberration induced from 120 h exposure to the dust because our preliminary data show no chromosome aberrations were observed after 24 h exposure. 120 h exposure to JSC-1A-f, JSC-1A-vf, Mars-1A, and UPM did not cause significant chromosome damage at tested concentrations. For example, at concentration of 50 µg/cm², JSC-1A-vf, Mars-1A, and UPM induced 3, 12, and 5% of metaphases with damage, respectively (Figure 3.A.); and 3, 13, and 5 total chromosome aberrations in 100 metaphases, respectively (Figure 3.B.). Mars-1A caused cell cycle arrest at concentrations of 100 µg/cm², and JSC-1A-vf caused cell cycle arrest at 50 and 100 µg/cm². Data were unable to be generated from higher concentrations due to abundant dust on the slides, which made the scoring inaccurate.

![Figure 1. Cytotoxicity of the Four Dusts to Human Skin Fibroblasts after 24 h Exposure. This figure shows lunar dust simulants, Mars dust simulant, and urban dust are cytotoxic to BJhTERT cells after a 24 hour exposure. The very fine lunar dust simulant and the Mars dust simulant were the most cytotoxic. For JSC-1A-f, concentrations of 50, 100, 200, and 400 µg/cm² are statistically different from control (p<0.05). For JSC-1A-vf, concentrations of 25, 100, 200, and 400 µg/cm² are statistically different from control (p<0.05). For Mars-1A, concentrations of 25, 50, 100, 200, and 400 µg/cm² are statistically different from control (p<0.05). For urban dust, concentrations of 50, 100, 200, and 400 µg/cm² are statistically different from control (p<0.05). Data represent an average of three experiments ± standard error of mean.](image-url)
Figure 2. Cytotoxicity of the Four Dusts to Human Skin Fibroblasts after 120 h Exposure. This figure shows lunar dust simulants, Mars dust simulant, and urban dust are cytotoxic to BJhTERT cells after a 120 hour exposure. The very fine lunar dust simulant and the Mars dust simulant are the most cytotoxic. For JSC-1A-f, concentrations of 100, 200, and 400 µg/cm$^2$ are statistically different from control (p<0.05). For JSC-1A-vf, concentrations of 100, 200, and 400 µg/cm$^2$ are statistically different from control (p<0.001). For Mars-1A, concentrations of 25, 50, and 100 µg/cm$^2$ are statistically different from control (p<.05). For urban dust, concentrations of 50, 100, 200, and 400 µg/cm$^2$ are statistically different from control (p<0.05). Data represent an average of three experiments ± standard error of mean.

Figure 3. Chromosome Damage of the Four Dusts to Human Skin Fibroblasts after 120 h Exposure. This figure shows that lunar dust simulants, Mars dust simulant, and urban dust do not significantly increase chromosome damage after a 120 hour exposure. Data represent an average of 3 experiments ± standard error of mean. Panel (A) shows the percent of metaphases with damage. Panel (B) shows the total chromosome damage in 100 metaphases. * Indicates no metaphases (50 and 100 µg/cm$^2$ for JSC-1A-vf and 100 µg/cm$^2$ for Mars-1A).
DISCUSSION

The risks of dust exposure are a major health concern for NASA as astronauts on planned lunar and Mars missions will encounter dusts of a variety of sizes and compositions. The present study aims to investigate the possible genotoxicity and cytotoxicity of lunar and Martian dust simulants, and urban dust to human skin fibroblast cells. Our study is important, as it is the first to investigate the potential carcinogenicity of lunar and Martian dust.

We found lunar dust simulants (JSC-1A-f, JSC-1A-vf), Mars dust simulant (Mars-1A), and urban dust are cytotoxic to human skin cells at concentrations of 25-400 µg/cm² (97-1, 565 µg/ml). All three dust simulants and urban dust caused a similar level of cytotoxicity after 24 h exposure, and JSC-1A-f is less cytotoxic than the other three dusts after 120 h exposure. Our data are consistent with other studies. Latch et al. (2008) reported lunar (JSC-1A) and Martian (Mars-1A) dust simulants decrease the viability of human alveolar macrophages with a concentration-dependent increase in apoptosis (100-500 µg/ml). Another study found Mars-1A reduces viability of HaCaT keratinocytes and is more cytotoxic than lunar dust simulants (JSC-A/B-1). At concentrations of 570-11, 360 µg/cm² lunar dust simulant induced only slight cytotoxicity in HaCaT keratinocytes (Rehders et al., 2011). The difference in toxicity could be due to the size difference. The size of JSC-A/B-1 (≤1 mm) used in their study (Rehders et al., 2011) is much bigger than those in the present work. We found Mars dust simulant, which has the smallest size, induces the highest cytotoxicity in skin cells among the four dusts after 120 h exposure. Interestingly, despite the similar particle size, urban dust is much less cytotoxic than Mars dust simulant, suggesting some chemical component of Mars dust simulant also played an important role in its toxicity. The most likely toxic components of Mars dust would be the chromium, titanium, or manganese compounds, or a combination of all three. We noticed JSC-1A-f did not cause an increase in cytotoxicity with increasing time, as the other three dusts did. The reason for this difference is not clear. A previous study reported lunar dust simulant (JSC-1A) at concentrations of 50-2000 µg/ml induced enhanced expression of inducible nitric oxide synthase (iNOS) in Murine Raw 264.7 Macrophage Cells (Chatterjee et al., 2010), indicating dust-induced reactive oxidation species may contribute to its cytotoxicity.

This study is the first to investigate the genotoxicity of lunar and Martian dust simulants. We found neither lunar, Martian dust simulants, nor urban dust, were clastogenic. Mars-1A and JSC-1A-vf caused cell cycle arrest at 100 µg/cm², indicating some DNA damage may have occurred, leading to the arrest. Studies have shown urban particulate matter induced DNA damage, including chromosome aberrations, DNA adducts, and strand breaks (Chen et al., 2013; Gutiérrez-Castillo et al., 2006; Healey et al., 2005). It should be noted the urban particulate matter used in those studies were collected at roadside with high-density of passing traffic, or in polluted areas containing a large number of genotoxic substances, and thus may explain why we don’t see any chromosomal aberrations in our urban particulate matter exposures.

In summary, our study shows extraterrestrial dust simulants are cytotoxic but not clastogenic to human skin cells. Their toxic effect is similar to the urban dust. Prolonged exposure to very fine lunar dust and Martian dust increases their cytotoxicity and induces some DNA damage. Given the extended time required for extraterrestrial exploration, the astronauts would be more likely chronically exposed to the dusts present there. Our data are important for understanding the potential health hazards astronauts may experience during planetary exploration. The data suggest prolonged exposure to extraterrestrial dusts may be dermally harmful to humans. It should be noted that many of the exact parameters encountered during a space mission were not replicated in these experiments (e.g., low gravity and microgravity, radiation), and these may consequently aggravate the effects of the dust. Furthermore, some of the physical properties of the actual extraterrestrial dusts are lost when using simulants (e.g., oxidized particle surface of Martian dusts; jagged and porous surface of lunar dusts).

ACKNOWLEDGEMENTS

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Sandra Wise for critical review of the manuscript. This work was supported by NASA grant # EP-08-01 (JPW), the Maine Center for Toxicology and Environmental Health, and the Department of Applied Medical Sciences, University of Southern Maine, Portland, ME.

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Lam CW, James JT, Latch JN, Hamilton RF Jr, Holian A (2002b) Pulmonary toxicity of simulated lunar and Martian dusts in mice: II. biomarkers of acute responses after intratracheal instillation. Inhalation Toxicology 14(9): 917-928
Wise et al. — Toxicity of Extraterrestrial and Urban Dusts in Human Skin Cells

**Research Article**

**Hydrogen- and Methane-Loaded Shielding Materials for Mitigation of Galactic Cosmic Rays and Solar Particle Events**

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**ABSTRACT**

One of the challenges of human spaceflight in deep space is the harsh radiation environment. The current best practices for mitigating radiation are via design and multifunctional materials. There have been many studies over the years showing low-Z materials as the best radiation mitigators for spaceflight. In addition, there have recently been several studies investigating hydrogen-loading of materials for fuel cells. If it is possible to load a material with additional low-Z materials — such as hydrogen — it may be possible to increase the radiation mitigating potential of these materials. Thus, our work is focused on metal hydrides (MHs), metal organic frameworks (MOFs), and nanoporous carbon composites (CNTs) that can be loaded with hydrogen or methane for radiation mitigation. Our previous simulation work focused on hydrogen-loading only, and investigated the capability of these materials during a particularly hard solar particle event (SPE) in October 1989. In these simulations, we found 50% of the investigated carbon composites outperformed high-density polyethylene (HDPE) — the current standard for passive radiation shielding. We also found 10% of the investigated MOFs outperformed HDPE. Therefore, we wanted to continue our simulation study of these materials to determine whether they may also show improvement over HDPE in a galactic cosmic ray (GCR) environment. Furthermore, there are concerns with using hydrogen as a loading material — a result of its flammability and instability in thermal extremes. Thus, we are also considering methane-loading of the MOFs and CNTs. The details of this work will be discussed in the paper. Overall, the results showed several MOFs, CNTs, and MHs that performed very well when compared with our typical spacecraft material of aluminum and our standard shielding material of HDPE. This study also showed there is little difference in the dose between hydrogen-loaded and methane-loaded materials of the same base chemistry.

**LIST OF ACRONYMS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>CNT</td>
<td>Nanoporous Carbon Composites</td>
</tr>
<tr>
<td>ESP</td>
<td>Energetic Solar Particle</td>
</tr>
<tr>
<td>EVA</td>
<td>Extravehicular Activity</td>
</tr>
<tr>
<td>GCR</td>
<td>Galactic Cosmic Ray</td>
</tr>
<tr>
<td>GEO</td>
<td>Geostationary Orbit</td>
</tr>
<tr>
<td>GLE</td>
<td>Ground Level Event</td>
</tr>
<tr>
<td>GTO</td>
<td>Geostationary Transfer Orbit</td>
</tr>
<tr>
<td>HDPE</td>
<td>High Density Polyethylene</td>
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<td>LEO</td>
<td>Low Earth Orbit</td>
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<tr>
<td>MEO</td>
<td>Medium Earth Orbit</td>
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<td>MH</td>
<td>Metal Hydride</td>
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<tr>
<td>MOF</td>
<td>Metal Organic Framework</td>
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<td>SPE</td>
<td>Solar Particle Event</td>
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</table>

**Key words:** Galactic Cosmic Rays; Solar Particle Events; Radiation Shielding; Metal Organic Framework; Metal Hydride; Nanoporous Carbon Composite; HZETRN; Deep Space

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INTRODUCTION

As the United States space program ventures out beyond the low Earth environment into what is known as “free space,” the risks from space radiation will increase substantially. The two sources of deep space radiation are the naturally occurring galactic cosmic radiation (GCR) and solar particle (proton) events (SPEs), both of which vary with the eleven-year solar cycle.

The solar cycle contains periods of high solar activity, known as “solar maximum,” and periods of low solar activity, known as “solar minimum” (Figure 1). During solar maximum, there tends to be more frequent and higher intensity SPEs, with the potential for very large events. The particles that make up these events consist of mostly protons.

The deep space GCR radiation environment consists of extremely energetic stripped nuclei ranging from hydrogen (proton) to iron with additional elemental nuclei out to uranium. The GCR environment varies with the eleven-year solar cycle such that during solar minimum the GCR fluxes are at a maximum, as seen in Figure 2.

There have been numerous radiation dose estimation studies (Atwell, 2012; Badavi et al., 2011; Cooper et al., 2008; Rojdev and Atwell, 2011; Simonsen and Nealy, 1991; Tripathi et al., 2006; Wilson et al., 2007) performed over the years for various types of space missions, both crewed and robotic. These missions include low Earth orbit (LEO), geostationary orbit (GEO), lunar, Mars, Jupiter/Europa, and Saturn. Additional mission analyses have also included extravehicular activity (EVA), medium Earth orbit (MEO), geostationary transfer orbit (GTO), and Earth orbit to deep space.

Passive, bulk shielding materials have been used to protect both crew and onboard systems since active shielding (e.g., magnetic and electrostatic) has not been feasible from a safety, weight, and cost standpoint. Evaluated shielding materials have included aluminum, hydrocarbons (e.g., polyethylene), hydrogen, methane, and other heavier materials (e.g., iron, tantalum, tungsten, and stainless steel) (Atwell et al., 2006; Atwell et al., 2013; Barghouty and Thibeault, 2006; Guetersloh et al., 2006; Rojdev et al., 2010; Walker et al., 2010). In general, materials that contain elements of a low atomic number provide better radiation mitigation properties than materials containing elements with a high atomic number. From previous investigations, HDPE has been identified as an efficient shielding material for space radiation applications due to the high hydrogen content. Thus, it is used as a reference material to which all other material results are compared in this work.

Figure 1. SPEs in accordance with the solar cycle (NASA, 2008). (This figure is from a NASA document and is not subject to copyright within the US.)
Recently, scientists have used certain materials loaded with hydrogen — namely MHs — to develop fuel cells having various applications, such as the automobile industry (Bowman and Fultz, 2002; Czaja et al., 2009; Iñiguez et al., 2004; Kuppler et al., 2009; Li et al., 2005; Mitrokhin, 2005; Sai toh et al., 2013; Sakintuna et al., 2007; Zhou et al., 2007). Since hydrogen, methane, and other types of hydrocarbons are excellent at shielding against proton radiation, we were curious whether the same materials being investigated for fuel cells could also be used for radiation shielding. In addition, we are most interested in those materials the investigators had difficulty extracting hydrogen that was loaded into the material, suggesting more stable bonding of the hydrogen. Therefore, we have investigated the use of three materials as potential radiation mitigators: hydrogen-loaded MHs, MOFs, and CNTs.

In a preliminary study (Atwell et al., 2014), we investigated 64 hydrogen-loaded materials and simulated their exposure to the series of SPEs that occurred during the 19-24 October 1989 time period. The combined differential and integral proton energy spectra for this series of events are shown in Figure 3. To arrive at this spectrum, the spaceflight data (Figure 4) was fitted using the Band fitting method (Atwell et al., 2010; Tylka et al., 2010). These proton spectra represent three Ground Level Events (GLE) plus one bow shock enhancement, as indicated in Figure 4. A GLE is of high enough energy such that neutron monitors on the surface of the Earth detect the secondary neutron production from the event. The energetic solar particles (ESP) occur when there is a bow shock enhancement of solar protons. We selected this series of events due to the particularly hard spectrum (high fluence of higher energy protons).

The materials studied were then compared against a typical spacecraft material of aluminum and our standard radiation shielding material of HDPE. The results (Table 1) showed over 60% of the materials studied performed better than aluminum (10 MOFs, 14 CNTs, and 15 MHs) and that these materials may be promising as multifunctional radiation shields. Thus, the work in this paper extends this initial investigation to the GCR environment, as well as compares the hydrogen-loaded materials with methane-loaded counterparts.
Figure 3. Integral and differential energy spectra for the SPEs occurring 19-24 October 1989, which exhibited a high fluence of higher energy protons (Tylka and Dietrich, 2009). (The data in this figure was provided by William Atwell for the referenced paper and he has given permission for replication here.)

Figure 4. Geostationary Operational Environmental Satellite system (GOES) satellite measurements of particle fluxes of various energies during the four SPEs of 19-24 October 1989. The times of the Ground Level Enhancements (GLE) and Energetic Solar Particles (ESP) are indicated on the plot. The ESP occurs when there is a bow shock enhancement of solar protons (NOAA, 2014). (The data in this figure is from the NOAA online database and is not subject to copyright within the US.)
Table 1. Results of a preliminary study (Atwell et al., 2014).

<table>
<thead>
<tr>
<th>Superior to HDPE</th>
<th>MOFs</th>
<th>CNTs</th>
<th>MHs</th>
<th>Total</th>
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<td>1</td>
<td>7</td>
<td>1</td>
<td>9</td>
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<tr>
<td>Inferior to Al</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

In the simulations undertaken in this study, a total of 85 materials were investigated. The material information was theoretically determined for the purposes of this study and these materials were separated into three categories: MOFs, CNTs, and MHs. The nanoporous carbon composites (CNTs) will most likely be made of carbon nanotubes initially when created and tested. The following three tables (Tables 2-4) provide the details of each material that were necessary for the radiation transport material definitions, as well as the radiation environment used for the exposure simulations (GCR or SPE).

Over the years, a number of high energy particle transport/dose codes have been developed. These include FLUKA (Ballarini et al., 2007), GEANT-4 (Bernabeu and Casanova, 2007), PHITS (Sihver et al., 2007), and HZETRN (Wilson et al., 1991; Wilson et al., 1995; Wilson et al., 2006). The first three codes are 3-D Monte Carlo codes and require long run times, as well as enhanced computing capacity. In our analyses we have used the HZETRN code, which is the NASA standard developed at NASA Langley Research Center. It is a one-dimensional, quick-running code that produces results comparable to the Monte Carlo codes.

For this study, we used the 2010 version of HZETRN (Wilson et al., 1991; Wilson et al., 1995; Wilson et al., 2006; Slaba et al., 2010a; Slaba et al., 2010b) to take advantage of the updates in the code. The environments specified for this study were the October 1989 series of SPEs (Figure 3 and Figure 4) and the 1977 solar minimum GCR condition. These environments are of interest because they represent worst-case environments. The October 1989 spectra was fit using the Band fitting method (Tylka and Dietrich, 2009), and the differential spectrum was used as the input SPE environment in HZETRN 2010 (Figure 3). The 1977 solar minimum GCR differential spectrum is pre-coded into the software (Figure 5). Output doses (cGy) in tissue were computed on a laptop at material thicknesses of 1, 5, 10, 20, 30, 50, and 100 g/cm², and a smooth line through these points was displayed.

RESULTS

There are two parts to this study. One is a continuation of work that was presented at the International Conference on Environmental Systems (Atwell et al., 2014) using the same groups of materials, but exposing them to a GCR environment. These materials are hydrogen-loaded MHs, MOFs, and CNTs.

The second part of the study focuses on methane-loaded materials because of potential concerns with hydrogen. One of the challenges with hydrogen-loaded materials is the stability of the hydrogen — especially in changing environmental conditions. When a vehicle is in space, engineers must design around several challenging environments, which include a changing thermal environment. Exposure of a hydrogen-loaded material to this environment could potentially lead to the hydrogen unbinding from the material and leaking into the spacecraft. Furthermore, hydrogen is known to be flammable and explosive, which could have devastating effects on spacecraft. Thus, given that methane is a radiation mitigator that performs better than polyethylene (Figure 6) and is less flammable than hydrogen, we investigated methane-loading of materials and compared it with the hydrogen-loaded version to determine the difference in dose vs. depth between the two loading methods. For this case, we investigated both the GCR environment and the SPE environment.
Table 2. MOF material formulas and densities used for radiation transport calculations and the simulated space radiation environment (“Exposure”) used. “Base” signifies the unaltered material, “H” is the hydrogen-loaded version, and “CH4” is the methane-loaded version. This information was provided by Drs. Daniel Liang, Matthew Hill, and Song Song.

<table>
<thead>
<tr>
<th>Loading Condition</th>
<th>Chemistry</th>
<th>Density (g/cm³)</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
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<tr>
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</tr>
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<td>GCR</td>
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<td>Base</td>
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<tr>
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<td>SPE, GCR</td>
</tr>
</tbody>
</table>

* MOFs are seen as a catch all phrase for periodic nanoporous materials. This is a non-metal, carbon-based framework that does not have the chemical structure of a CNT, has similar properties to MOFs, and large adsorption capacity.
### Table 3. CNT material formulas and densities used for radiation transport calculations and the simulated space radiation environment (“Exposure”) used. “Base” signifies the unaltered material, “H” is the hydrogen-loaded version, and “CH4” is the methane-loaded version. The subscripts give the mole percent of each radical in the group. This information was provided by Drs. Daniel Liang, Matthew Hill, and Song Song.

<table>
<thead>
<tr>
<th>Loading Condition</th>
<th>Chemistry</th>
<th>Density (g/cm³)</th>
<th>Exposure</th>
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<tbody>
<tr>
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</table>

### Part 1: H-Loaded Materials Exposed to a Simulated GCR Environment

The first part of the study focused on hydrogen-loaded materials that were exposed to the input GCR environment. The results are presented by material class: MOFs, CNTs, and MHs. The materials are also compared with aluminum (i.e., our typical spacecraft material) and HDPE (i.e., our standard radiation shielding material).

**Metal organic frameworks**

The results of the MOF materials are shown in Figure 7 and Figure 8. In these results, we see all MOF materials perform better than the typical spacecraft material of aluminum. In all cases, the hydrogen-loaded versions perform better than the non-hydrogen-loaded versions. Additionally, none of the MOFs are better mitigators than HDPE. However, there are two hydrogen-loaded MOFs that perform similarly to HDPE, namely C₂₀₀H₃₂₅ and C₄₃₂H₁₁₂₀Be₄₄O₁₄₄.
Table 4. MH material formulas and densities used for radiation transport calculations and the simulated space radiation environment ("Exposure") used. “Base” signifies the unaltered material and “H” is the hydrogen-loaded version. This information was provided by Drs. Daniel Liang, Matthew Hill, and Song Song.

<table>
<thead>
<tr>
<th>Loading Condition</th>
<th>Chemistry</th>
<th>Density (g/cm³)</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>Li₂.₃₅Si</td>
<td>1.67</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>91% Li₂.₃₅Si and 9% H</td>
<td>0.84</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>LiB</td>
<td>1.65</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>91% LiB and 9% H</td>
<td>0.67</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>CaNi₅</td>
<td>6.60</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>96% CaNi₅ and 4% H</td>
<td>6.6</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>LaNi₄.₇Al₀.₃</td>
<td>8.00</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>96% LaNi₄.₇Al₀.₃ and 4% H</td>
<td>7.6</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>LaNi₄.₈Sn₀.₂</td>
<td>8.40</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>96% LaNi₄.₈Sn₀.₂ and 4% H</td>
<td>8.4</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>LaNi₅</td>
<td>8.20</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>LaNi₅H₆</td>
<td>6.22</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>Al₂Cu</td>
<td>5.83</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>Al₂CuH</td>
<td>5.39</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>Al</td>
<td>2.70</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>AlH₃</td>
<td>2.5</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>BaAlH₃</td>
<td>3.30</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>SrAl₂H₂</td>
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<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>Ti₀.₉₈Zr₀.₀₂V₀.₄₈Fe₀.₈₉Cr₀.₀₉Mn₁.₅</td>
<td>7.20</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>Ti₀.₉₈Zr₀.₀₂V₀.₄₈Fe₀.₈₉Cr₀.₀₉Mn₁.₅H₃.₃</td>
<td>5.80</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>TiCr₁.₈</td>
<td>5.70</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>TiCr₁.₈H₃.₅</td>
<td>4.50</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>TiFe₀.₉Mn₀.₁</td>
<td>6.50</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>TiFe₀.₉Mn₀.₁H₂</td>
<td>5.20</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>LiAlH₄</td>
<td>0.92</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>LiMg(AlH₄)₃</td>
<td>1.80</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>Mg(AlH₄)₂</td>
<td>2.24</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>NaAlH₄</td>
<td>1.81</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>Y₃Al₂H₆.₅</td>
<td>4.10</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>V</td>
<td>6.00</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>VH</td>
<td>5.60</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>VH₂</td>
<td>2.30</td>
<td>GCR</td>
</tr>
<tr>
<td>Base</td>
<td>Li</td>
<td>0.53</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>80% Li and 20% H</td>
<td>0.57</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>85% Li and 15% H</td>
<td>0.56</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>90% Li and 10% H</td>
<td>0.55</td>
<td>GCR</td>
</tr>
<tr>
<td>H</td>
<td>95% Li and 5% H</td>
<td>0.54</td>
<td>GCR</td>
</tr>
</tbody>
</table>
Figure 5. Differential spectrum of the 1977 solar minimum GCR environment pre-coded into HZETRN. For ease of viewing, only the protons are plotted. The code includes a total of 39 species for the GCR environment.

Figure 6. SPE dose as a function of depth for liquid hydrogen, liquid methane, aluminum, and HDPE (Atwell et al., 2014). The input environment for this calculation is the Band fit of the October 1989 series of events (Figure 3), and the resultant data presented in this figure is from a simulation performed with HZETRN 2010 (Wilson et al., 1991; Wilson et al., 1995; Wilson et al., 2006; Slaba et al., 2010a; Slaba et al., 2010b).
Figure 7. GCR absorbed dose curves for three MOF materials and their hydrogen-loaded counterparts compared with aluminum (red) and HDPE (black). The hydrogen-loaded versions are denoted by a dashed line and a filled in marker. The non-hydrogen-loaded MOF is denoted by a solid line and an open marker.

Figure 8. GCR absorbed dose curves for two additional MOF materials and their hydrogen-loaded counterparts compared with aluminum (red) and HDPE (black). The hydrogen-loaded versions are denoted by a dashed line and a filled in marker. The non-hydrogen-loaded MOF is denoted by a solid line and an open marker.
Nanoporous carbon composite

The results for the CNTs are shown in Figure 9 and Figure 10. The non-loaded versions (Figure 9) are better radiation mitigators than aluminum. While the non-loaded CNTs do not outperform HDPE as a radiation mitigator, they are very similar. The hydrogen-loaded versions of these CNTs (Figure 10) outperform aluminum again and just slightly outperform HDPE.

Metal hydrides

The results for the various MHs investigated are shown in the following figures: Figure 11 shows a series of lithium MHs with increasing hydrogen content and decreasing lithium content, Figure 12 shows additional MHs, and Figure 13 shows two lithium-based materials and their hydrogen-loaded counterparts.

All of the lithium-containing materials consistently outperform aluminum and the lithium MHs either outperform both aluminum and HDPE, or are in line with HDPE. The other investigated MHs only surpass aluminum in their radiation mitigation traits.

There were also 25 additional materials investigated (not shown in the graphs) that performed worse than aluminum.

Figure 9. GCR absorbed dose curves for seven non-hydrogen-loaded CNTs compared with aluminum (red) and HDPE (black).

Figure 10. GCR absorbed dose curves for seven hydrogen-loaded CNTs compared with aluminum (red) and HDPE (black).
Figure 11. GCR absorbed dose curves for five hydrogen-loaded lithium MHs compared with aluminum (red) and HDPE (black).

Figure 12. GCR absorbed dose curves for five hydrogen-loaded MHs compared with aluminum (red) and HDPE (black).
Part 2: CH₄-Loaded Exposed to Simulated GCR and SPE Environments

In the second part of the study, we investigated several MOFs and CNTs. We compared the unloaded versions to the hydrogen-loaded and methane-loaded versions, as well as to our typical spacecraft material of aluminum and our standard shielding material of HDPE. For these materials, we investigated both the SPE and the GCR environments.

**SPE**

The following graphs show the results of various MOF materials exposed to the October 1989 series of SPEs. Figure 14 and Figure 15 show five materials and their respective hydrogen-loaded and methane-loaded versions. In each of the cases, the hydrogen-loaded version outperforms the base and the methane-loaded versions. However, the methane-loaded version is quite comparable to the hydrogen-loaded version. Additionally, all of the MOF materials outperformed aluminum in their radiation mitigation qualities.

Figure 16, Figure 17, and Figure 18 are the results of the CNTs exposed to the same October 1989 series of SPEs. For these materials, all three cases (base material, hydrogen-loaded, and methane-loaded) are very similar. All the materials perform better than aluminum and only the hydrogen-loaded versions outperform HDPE. However, they are all relatively close to the performance of HDPE.

**GCR**

Figure 19 and Figure 20 show the results of the MOF materials exposed to a GCR environment. In these results there are a few of the base materials that perform worse than aluminum. However, the hydrogen- and methane-loaded counterparts surpass the radiation mitigation.
Figure 14. SPE absorbed dose curves for three MOFs compared with their hydrogen-loaded and methane-loaded versions, as well as aluminum (red) and HDPE (black). The base MOF is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a filled marker, and the methane-loaded version is depicted by a dotted line and an open marker.

Figure 15. SPE absorbed dose curves for two MOFs compared with their hydrogen-loaded and methane-loaded versions, as well as aluminum (red) and HDPE (black). The base MOF is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a filled marker, and the methane-loaded version is depicted by a dotted line and an open marker.
Figure 16. SPE absorbed dose curves for three CNT materials compared with their hydrogen- and methane-loaded versions, as well as compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a filled marker, and the methane-loaded version is depicted by a dotted line and an open marker.

Figure 17. SPE absorbed dose curves for two CNT materials compared with their hydrogen- and methane-loaded versions, as well as compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a filled marker, and the methane-loaded version is depicted by a dotted line and an open marker.
Figure 18. SPE absorbed dose curves for two CNT materials compared with their hydrogen- and methane-loaded versions, as well as compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a filled marker, and the methane-loaded version is depicted by a dotted line and an open marker.

Figure 19. GCR absorbed dose curves for three MOF materials and their hydrogen-loaded and methane-loaded counterparts, compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and closed marker, and the methane-loaded version is depicted by a dotted line with an open marker.

qualities of aluminum. Only one hydrogen-loaded MOF outperforms HDPE, namely Zn$_{216}$C$_{3132}$O$_{702}$H$_{14814}$.

Figure 21, Figure 22, and Figure 23 show the results of the CNTs exposed to the GCR radiation environment. The CNT materials again behave similarly to HDPE — as was shown with the SPE environment—and thus, outperform aluminum as a radiation mitigating material. There are a few hydrogen-loaded CNT materials that also outperform HDPE.
Figure 20. GCR absorbed dose curves for two MOFs and their hydrogen-loaded and methane-loaded counterparts, compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and closed marker, and the methane-loaded version is depicted by a dotted line with an open marker.

Figure 21. GCR absorbed dose curves for three CNTs and their hydrogen-loaded and methane-loaded counterparts, compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a closed marker, and the methane-loaded version is depicted by a dotted line and an open marker.
Figure 22. GCR absorbed dose curves for two CNTs and their hydrogen-loaded and methane-loaded counterparts, compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a closed marker, and the methane-loaded version is depicted by a dotted line and an open marker.

Figure 23. GCR absorbed dose curves for two CNTs and their hydrogen-loaded and methane-loaded counterparts, compared with aluminum (red) and HDPE (black). The base material is depicted by a solid line, the hydrogen-loaded version is depicted by a dashed line and a closed marker, and the methane-loaded version is depicted by dotted line and an open marker.
DISCUSSION

There are two objectives in this study. The first objective is to evaluate these types of materials against our typical spacecraft and shielding materials to determine whether they may be viable as multifunctional materials that also protect against space radiation. The second objective is to determine whether the methane-loaded versions of these materials are comparable to the hydrogen-loaded versions to remove some of the concerns in working with hydrogen.

To determine how these materials fare against our typical spacecraft materials, the dose results have been compared with aluminum (the typical spacecraft shell) and HDPE (our standard radiation shield). We have aggregated the data of the 86 materials investigated and separated them out by the following categories: materials that perform better than HDPE, materials that perform better than aluminum but not better than HDPE, and materials that do not perform better than aluminum. These comparisons were made at each of the thicknesses investigated. The aggregated data are shown below in Table 5 and Table 6.

Table 5. Aggregated data of materials exposed to a SPE and how they compare with a typical spacecraft material (aluminum) and the standard radiation shielding material (HDPE).

<table>
<thead>
<tr>
<th></th>
<th>MOFs</th>
<th>CNTs</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-loaded</td>
<td>H-loaded</td>
<td>CH4-loaded</td>
<td>non-loaded</td>
<td>H-loaded</td>
<td>CH4-loaded</td>
<td>non-loaded</td>
<td>H-loaded</td>
<td>CH4-loaded</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior to HDPE</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Al and HDPE</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior to Al</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Aggregated data of materials exposed to a GCR and how they compare with a typical spacecraft material (aluminum) and the standard radiation shielding material (HDPE).

<table>
<thead>
<tr>
<th></th>
<th>MOFs</th>
<th>CNTs</th>
<th>MHS</th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-loaded</td>
<td>H-loaded</td>
<td>CH4-loaded</td>
<td>non-loaded</td>
<td>H-loaded</td>
<td>CH4-loaded</td>
<td>non-loaded</td>
<td>H-loaded</td>
<td></td>
</tr>
<tr>
<td>Superior to HDPE</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Between Al and HDPE</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>Inferior to Al</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>16</td>
<td>28</td>
</tr>
</tbody>
</table>

From these tables, it is quite clear a majority of these materials do outperform aluminum as a shielding material, and a few also outperform HDPE. Of particular interest are the lithium-based MHs that outperformed HDPE, as shown in Figure 11, Figure 12, and Figure 13. Further work will be needed to determine why this occurs.

The other group of materials that performed particularly well are the CNTs. Both the non-loaded and the methane-loaded versions of the materials performed better than aluminum, and while they did not perform better than HDPE, they are comparable (Figure 9, Figure 16, and Figure 23). The hydrogen-loaded versions of the CNTs outperformed HDPE, but only marginally. The reason the results of the CNTs are so similar to HDPE is their chemical makeup is very similar and thus, they have similar radiation shielding characteristics.

There were also several MHs that did not outperform aluminum. These materials tended to have elements with higher atomic numbers —
such as lanthanum, barium, calcium, etc. It is well known from previous radiation shielding studies (Atwell et al., 2006; Atwell et al., 2013; Barghouty and Thibeault, 2006; Guetersloh et al., 2006; Walker et al., 2010; Wilson et al., 1997) that better radiation mitigators will have a smaller atomic number. One caveat is certain materials —such as lithium and boron — are known to be good neutron absorbers while also containing a higher atomic number than hydrogen. Thus, optimizing a material to reduce the primary radiation energy enough to stop the particle, as well as providing some neutron absorption from secondary radiation production, may be the better option for a radiation shield.

In addition to determining the radiation shielding capability of these materials relative to our typical spacecraft materials, we wanted to determine whether the methane-loaded versions were comparable to the hydrogen-loaded versions. Therefore, we calculated the percent difference in dose between the hydrogen-loaded versions and the methane-loaded versions for a thickness of 30 g/cm² using Equation (1). In the equation, $X_{CH_4}$ is the dose for the methane-loaded version and $X_H$ is the corresponding dose for the hydrogen-loaded version. The differences are shown in Table 7 and Table 8.

$$\%\ increase\ in\ dose = \frac{X_{CH_4} - X_H}{X_H} \times 100$$

Table 7. The percent increase in dose for the methane-loaded MOF materials compared with the hydrogen-loaded equivalents for both the SPE and GCR cases. The comparisons were made for a thickness of 30 g/cm².

<table>
<thead>
<tr>
<th>Base Material</th>
<th>MOF</th>
<th>CH4 dose higher than H</th>
<th>SPE</th>
<th>GCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn$<em>{216}$C$</em>{3132}$O$<em>{702}$H$</em>{1242}$</td>
<td>34%</td>
<td>12%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$<em>{1536}$H$</em>{864}$Cu$<em>{96}$N$</em>{32}$O$_{480}$</td>
<td>3%</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$<em>{288}$H$</em>{96}$Cu$<em>{48}$O$</em>{240}$</td>
<td>0%</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$<em>{112}$C$</em>{192}$O$<em>{128}$Zr$</em>{12}$Ti$_{12}$</td>
<td>2%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$<em>{112}$C$</em>{192}$O$<em>{128}$Zr$</em>{24}$</td>
<td>1%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. The percent increase in dose for the methane-loaded CNT materials compared with the hydrogen-loaded equivalents for both the SPE and GCR cases. The comparisons were made for a thickness of 30 g/cm².

<table>
<thead>
<tr>
<th>Base Material</th>
<th>CNT</th>
<th>CH4 dose higher than H</th>
<th>SPE</th>
<th>GCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{97.7}$C$</em>{2.30}$</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{93.7}$C$</em>{6.73}$</td>
<td>1%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{89.06}$C$</em>{10.94}$</td>
<td>2%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{79.41}$C$</em>{20.59}$</td>
<td>4%</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{63.16}$C$</em>{36.84}$</td>
<td>8%</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{50}$C$</em>{50}$</td>
<td>12%</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C$<em>2$H$<em>4$)$</em>{39.13}$C$</em>{60.87}$</td>
<td>17%</td>
<td>6%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In examining these tables, it is quite clear there is minimal difference in the dose between the methane-loaded and hydrogen-loaded versions of these materials, with the exception of $\text{Zn}_{216}\text{C}_{3132}\text{O}_{702}\text{H}_{1242}$ during the SPE exposure. It is unclear at this time why there is such a departure between the hydrogen-loaded and methane-loaded version of this material, and future study will be needed. Furthermore, in the GCR cases there tends to be even less of a difference between the hydrogen- and methane-loaded versions.

CONCLUSIONS

This study examined several hydrogen- and methane-loaded materials to determine whether they would be feasible multifunctional materials that could serve as radiation shields in space. Furthermore, there are general safety concerns about using hydrogen due to its flammability and instability in changing environments. Thus, we considered methane as an alternative loading material and compared its radiation shielding performance against the hydrogen-loaded counterpart.

Overall, the results showed several MOFs, CNTs, and MHs that performed very well when compared with our typical spacecraft material of aluminum and our standard shielding material of HDPE. Of particular interest are the lithium MHs that outperformed HDPE. It is recommended future studies investigate additional lithium-based materials and perform deeper investigation to determine why these materials perform so well. Additionally, CNTs have similar chemical composition to HDPE and thus provide similar radiation protection. Since HDPE tends to be a parasitic shielding material due to its poor structural performance and flammability concerns, it is recommended future studies also include CNTs that may have multifunctional structural uses as well. Future work will also need to consider dose equivalent and other exposure quantities of interest for human spaceflight.

Finally, this study showed there is little difference in the shielding effectiveness between hydrogen-loaded and methane-loaded materials of the same base chemistry. Thus, future studies should focus on methane-loaded materials to remove some of the safety concerns in using hydrogen.

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