Exploring the Microbiome/ Immunome and Disease on the International Space Station

IMPROVING HUMAN HEALTH ON EARTH

CASIS

Consolidation of Requirements and Group Concurrence

Group 1: The National Microbiome Initiative and interagency research on ISSNL

Session Lead: Elizabeth Stulberg, Ph.D, USDA

To promote interagency research on the ISSNL, STEM education programs are a good collaboration point. In addition to working with other agencies, agencies can work with schools and youth groups. Education needs to start early with K-12 programs.

Many agencies involved in the National Microbiome Initiative (NMI) hold their own mission-oriented meetings, reviews and conferences (e.g. NIH, NIST). To promote collaboration between agencies, and to get the word out to the scientific community that the ISS is an available resource, stakeholders need invitations to these meetings and conferences. Exchanging ideas at meetings and conferences will foster interactions and inform on the uses of microgravity in research.

In addition to agency conferences and workshops, which colleagues should share widely with stakeholders, sessions on cross-cutting microbiome research that include microgravity could be proposed for annual meetings of scientific societies.

Group 1 identified research that can benefit Earth and astronauts and broke that research down into two types: that which requires microgravity and that which does not.

Microbiome research that does not require microgravity includes the study of lightweight shelf stable meals, data management, energy production/bioreactors, and diagnostics.

Microbiome research that does require microgravity includes the study of relevant disease models, accelerated antibiotic resistance, protein structure, the built environment (e.g., closed systems like the ISS),using microbes for processes such as cleaning (e.g. reclamation, filtration, and CO2 mitigation), the stability of microbiomes in space, food systems (e.g., plant growth), investigation of cryptic pathways for bacterial production of products, and systems biology projects.

Group 1 also identified the need for a paradigm shift. Government agencies are to fund their missions, and only their missions. Microbiome research is cross cutting and not confined to an agency. The challenge is to obtain funding for research that may include the mission of more than one agency.

Group 1 Recommendations

- Collaboration
  - Stakeholders need invitations to attend meetings and conferences
  - Pursue partnerships/collaborative agreements with agencies that have complementary missions
  - Recommend to the National Microbiome Initiative (NMI) government funding of research that covers the missions of more than one agency
- Research Topics
  - Microbiome projects that do not require microgravity
    - Light weight shelf stable meals
    - Data management

        - Energy production/bioreactors
        - Diagnostics
    - Microbiome projects that require microgravity
        - Study of relevant disease models
        - Accelerated antibiotic resistance
        - Protein structure
        - The built environment (e.g., closed systems like the ISS)
        - Using microbes for cleaning (e.g. reclamation, filtration and CO2 mitigation)
        - Stability of microbiomes in space
        - Food production systems (e.g., plants)
        - Investigation of cryptic pathways for bacterial production of products
        - Systems biology projects

Group 2: Microbiology of the built environment: Earth and Space

Session Lead: Julie Robinson, Ph.D., NASA-JSC

Dr. Robinson started off by highlighting three ISS experiences, 1. fungus 2. non-specific rashes and 3. biofilms. Fungus is particularly interesting as it can be detrimental to both human health and the ISS via corrosion. Fungus ended Mir and to prevent the same from happening on the ISS it is kept dry. However, if the ISS gets excessively moist or on long space missions such as to Mars, fungus may be more of a factor.

Non-specific rashes are being studied in the context of astronaut immune dysfunction and in the context of a closed environment as part of the Astronaut Microbiome Project. Astronauts have had to take steroids because of the rashes. The closed system, while it may act like a "sick building" in that new air is never brought in, it also advantageous for study in its simplicity and small numbers of people. Sampling rashes on mission would provide additional information to use in diagnoses. To reach statistical significance the few astronauts should be repeatedly sampled in longitudinal studies, as is being done with the Astronaut Microbiome Project.

Biofilms are of interest because there is some evidence that they are more prone to form in space. Both the symbiotic and pathologic potentials of biofilms were discussed. The ISS offers different fluid physics from earth which may affect quorum sensing and when and how biofilms form. This information could help us to prevent unwanted biofilms or perhaps produce biofilms with beneficial characteristics such as UV resistance or antifungal properties.

Group 2 also discussed how the ISS is both similar to and different from Earth-bound built environments and how this can shape research. Submarines have many technologies in common with the ISS and are probably one of the ISS's best Earth analogs. The ISS is also similar to other built environments such as ICUs and offices in terms of air recirculation. We should develop an understanding of how closely these built environments resemble each other and why. Built environment microbiome research shares the challenges of discerning whether genetic material is from dead or alive organisms and understanding the impact of horizontal gene transfer. We cannot eliminate the possibility that DNA from dead organisms may affect the system through transduction.

To make research applicable in between groups it was suggested that research be standardized, using 16s rRNA, and also likely 18s rRNA, as a start. Standardized protocols and automation are desired to remove variation in extraction protocols and sampling methods. When sampling the ISS for built environment studies, that sampling should be modeled on ICU studies, where sampling is extensive and infrequent. This data

could then more easily be compared to data from other built environment studies. This could be a supplement to the current procedure where ISS surface sampling is less extensive.

One research idea was to test automated systems on the ISS, exploiting the fact that it is a closed system. Astronauts could introduce benign microbes or "barcoded" DNA particles into the ISS environment, then evaluate the automation system's detection of same. It was also suggested that germ-free model organisms such as mice could be later incorporated.

Space also offers unique challenges and opportunities, which can still benefit both Earth and Space. The unique environment of the ISS includes microgravity, low shear, increased radiation, and lack of convection and buoyancy. More extensive research into the following was proposed: particle size, as microgravity may affect this, differences in cell division, phage studies including host and pathogen interactions and environmental and stress studies given that spaceflight can simulate aging.

In addition to internal culturing and sampling, experiments external to the ISS can also be performed. MISSE FF is a robotically serviced, external exposure facility that doesn't require a spacewalk to access it. Other external facilities are also available. Reasons to sample outside the ISS include research on organisms able to resist a vacuum and research on radiation resistant bacteria and microorganisms that might produce a UV-resistant pigment. Such a pigment could have numerous applications, including civilian defense.

Group 2 Recommendations

- Study
  - Fungus
  - Rashes as relate to
    - Immune dysfunction
    - The closed environment
    - Possible causative organisms such as reactivation of the virus causing zoster
  - The ISS as a closed system and built environment
  - Biofilms
    - Potentials
      - Symbiotic
      - Pathologic
    - Formation in space
- Standardize Research
  - 16s rRNA and 18s rRNA
  - Automation
  - Standardized protocols
  - When studying the ISS as a built environment, sample extensively and infrequently
  - Repeatedly sample astronauts
- Research Topics
  - Internal
    - Automated systems
    - The Introduction of benign microbes or DNA particles to the ISS
    - Germ free mice
    - Particle size
    - Cell division
    - Phage studies
    - Host and pathogen interactions
    - Environmental and stress studies
  - External
    - MISSE FF
    - UV resistant microorganisms
    - UV resistant pigments
    - Organisms able to resist a vacuum

Group 3. Microbiology of the Human Environment.

Session Lead: Mark Ott, Ph.D., NASA-JSC

Group 3 addressed one of the fundamental question of microbiome research, what is a healthy microbiome. It is known that the microbiome affects aging, behavior, oral health and the immune system among other things. We also know that the interaction is a two-way street and that microbiome fitness can be affected by environmental changes such as dietary choices. To research these and other microbiome concepts on the ISS the group discussed the specifics of research subjects, data gathering and controls.

Microbiome research subjects can include the astronauts themselves, rodents, and other model systems. The current ISS astronaut sample size is nine. Because nine is a small sample size the group determined as much data as possible should be gathered from these individuals including dietary, medication and mood

data. Feces, urine, saliva, blood, skin and environmental samples could also be frozen or fixed to later analyze RNA for 'omics data to see which parts of the microbiome are functional. (Rodents can also serve as research subjects and sampling should include their feces and the areas around and in their cages.) Mood data could be quantified with a mental skill tests such as the one-minute type to assess cognitive ability. The astronauts could serve as their own controls by having data gathered pre-, post-, and during flight. Citizen scientists could also be called to contribute samples and thus serve as another control group.

Both rodents and humans experience increased stress on the ISS. The Navy, Air Force, Army and DARPA are interested in understanding microbiome modulation in a stressful environment. There is the potential to form collaborations across multiple agencies including NASA, DOD, USDA and NIH.

Group 3 Recommendations

- Research subjects
  - Astronauts
  - Rodents
  - Other model systems
- Data
  - Obtain as much as possible
  - Sources
    - Feces
    - Urine
    - Saliva
    - Blood
    - Skin
    - The environment
  - Evaluation
    - Freeze or fix specimens
    - Evaluate RNA later for 'omics data
    - Quantify psychological data with mental skills test
- Controls
  - Astronauts themselves
  - Citizen scientists
- Potential Collaborations
  - DOD
  - Multiple Agencies including NASA, DOD, NIH, etc.

Group 4. What's Next for Microbiome Research in Space?

Session Lead: Michael Roberts, Ph.D., CASIS

The ISSNL is a tool and CASIS' goal is that it be used for earth benefit. Potential questions would be how does long term isolation in a closed system affect the microbiome and how does the microbiome impact health. The purpose of the workshop is to identify potential investigations and partnerships for microbiome research that exploit the unique capabilities of the ISS.

There are eight more years for experimentation on the ISS. There is the possibility to "piggyback" on research already going on the ISS including viral transfer, latent virus activation, rodent models, and the microbiome's effects on wound healing in the rodent models. Most experiments have overlooked the microbiome, so there is opportunity to collect microbiome data from these studies.

Rodent research is limited to 40 mice and NASA has more experience with female mice than male mice. The hardware that can support animal husbandry study is on orbit for six months. The rodent research experience is 60 days. Ground control uses the same hardware as ISS and the control animals have the same carbon dioxide, humidity, temperature, and lighting cycle. The mice are the same cohort matched for weight.

At present there are not enough replicates to get statistical significance for microbiome samples from the astronaut project. CASIS is interested in sample size and determining for rodent microbiome studies whether it is better to continue research on current subjects or move to genetic diversity. Dr. Voorhies commented on the need for both. However, if only one were an option he would choose more subjects over genetic variation. Studies could initially determine how microgravity affects one genotype then be expanded. An advantage of genetic variation is knowing that the response isn't the peculiarity of one inbred strain. The tradeoff off genetic diversity is decreased sample size, going from 40 mice to five or 10.

Dr. Turek doesn't recommend cultured cross strains because he does not see the diversity as an advantage. Genetic tools can't be used with the random combinations of genes of genetically diverse mice. He uses strains that respond differently to stress. He would continue using the inbred strains. Therefore, the migration viewpoint, rather than the genetic diversity viewpoint, would also be the best angle for ISS research. However, Dr. Chrisey, Office of Naval Research points out that Dr. Janssen at PNNL/LBNL has found that one advantage of cross-collaborative mouse models can be the potential to identify mouse genome loci that respond to changes in the gut microbiome.

The group discussed studies that looked at the microbiome in breeding facilities. The ISS is likely the ultimate in a controlled breeding environment, yet the microbiome changed between inbred mice strains. The microbiome population is not homogeneous. How the animals are caged and whether their feces are removed from the cage will affect how homogenous the population can get. Coprophagy enables cross-inoculation. This needs to be kept in mind when analyzing the experimental effects of microgravity or stress.

Both DARPA and NIH are working on chips. DARPA is working on body on a chip and NIH is working on single or dual organ systems. The ultimate goal is to replace animal models. How could the chips be used to study the innate immunity of the cells? Could you measure a response to a viral analog? The tissue on a chip expresses biomarkers and pharmaceutical are interested for drug testing. CASIS currently has two tissue-on-chip projects in development for flight. Each investigator awarded a grant has two flight opportunities.

Cancer progression is an evolutionary problem. Some cancer derived cell lines quickly differentiate. The group discussed that cancer models in mice have been put on the ISS and they did progress faster. The two models to study tumor growth in microgravity are:

1. Using four or five cell types to study replication speed and tumor size.
2. Using one strain of mice in a transplantation model.

There is the potential to look at cancer causing viruses and simulating oncogenesis in a mouse model. There was interest in how preadaptation to cancer, versus non cancer, cells, or both, allow viruses to track or not, cancer progression. Dr. Roberts noted that telomerase doesn't work as well in microgravity, there's strong evidence T cells are quickly impaired in microgravity and there are large changes in cardiac function.

Dr. Turek would like to see how bone loss and muscle atrophy are affected by the combination of stress and the microbiome on the ISS. Pharmaceutical companies may want to know the effect of their drug in a zero gravity environment. He'd also like to do an experiment with a drug or probiotic that targets the microbiome community associated with a tumor. He would like more data for circadian medicine and precision medicine and is interested in an implanted chip to gather data. Others responded that blood pricks such as those done by diabetics could also provide data.

The group also addressed experiments that can be done on the ISS that can't be done on Earth considering the ISS is a truly closed environment other than crew changes or resupply missions. It was suggested a dose release and recapture experiment could be done where *we* radiolabel a non-pathogenic organism to see how it spreads. Radiation studies could be done to look at whether radiation has the same effects in microgravity as on earth. It is known radiation on the ISS is 10 – 100 fold higher than on Earth and the mutation rate is slightly elevated.

DARPA is interested in survival in extreme environments. DARPA would like to study how some molecules or proteins might crystallize only in space. They would like to bring bacteria to the ISS to see if they produce never before seen molecules and to see what their metabolome looks like. Radiation resistant genes from the tardigrade (water bear) have been inserted into human stem cells. Is it possible to incorporate radiation resistance into organisms such as a mouse then test them on the ISS? Can we load a gene onto an AAV vector and transiently express radiation resistance? This would be useful for time in space or a nuclear event. Can we make bacteria that convert fecal material or leftover food into useful products? What automated or remote controlled experimental tools can we make? Regarding prototrophy can we add to microbes the ability to make nutrients, such as vitamin D, that we can't produce on our own? Can we lower cost of growth media by doing pharmacologic studies on mammalian cells?

CASIS wanted the group's opinion on whether hardware automation or citizen scientists are preferred for the ISS. Having a scientist increases the scientific return, however it takes crew time to run the analysis, experiments are more difficult, and there are time and training limitations. DARPA and Dr. Voorhies prefer automation and bringing that capability to space.

Dr. Roberts explained that there is a crew of six: two U.S., one international and three Russian. The seventh added astronaut will be American increasing research time from 40 to 80 hours a week. There are only two vehicles that take samples up and return. The Russian Soyuz can take 15kg of samples and Space X's Dragon will go up three to four times this year. In addition there is a new commercial resupply contract that will increase frequency to every three months. This vehicle will have wings enabling it to land. This will change study design as it's an advantage for getting rodents back and it will decrease the time to get samples from Space to one hour.

Also new this year to ISS is quantitative PCR, supporting infrastructure to move fluids, nucleic acid extraction and ability to generate sequence data. RNA can be shipped back now, but previously analyses needed to be done on ISS as the RNA half-life is about five minutes and the sample would change in the amount of time it would take to return to Earth.

CASIS also works with international companies on the microbiome. Companies can have research sent up, they have identified common goals and are amenable to sharing biospecimens. The relationship between drug efficacy, pharmacokinetics and pharmacodynamics and the microbiome can be studied.

In summary, Group 4 discussed opportunities that can be found through accessing the ISS. The ISS is a closed system, with microgravity and increased radiation. It is a stressful environment and studies can be done to learn more about how our microbiome responds to these stressors and what effects that can have on human health.

The group primarily focused on how human health and the microbiome are affected by the stressful environment of Space. They also identified the opportunity to harness microbiome capabilities for our own good and the development of quick portable research systems that can be used in Space as well as extreme Earth environments.

Group 4 Recommendations

- Research topics
    - Phages to combat multi drug resistance.
    - Microbiome assembly and sources. Is gravity a constraint in microbiome assembly?
    - How do microbiomes change on the ISS and then again on Earth. Do they revert, stay the same, or become something new?
    - Lenski (*E. coli* long-term evolution experiment (LTEE)) experiments.
    - Biofilms, considering the biofilm structure change exhibited by Aeruginosa.
    - How the low shear force of the ISS affects viral oncogenesis.
    - The interplay between worsening innate immunity on the ISS and increased pathogenesis of certain organisms.
    - How the interrupted circadian rhythm aboard the ISS affects the microbiome.
    - Assessing the effect of the ISS's stressful environment on transference of the microbiome from parent to offspring. Could mice be bred on the ISS to follow microbiome development?
    - Using the microbiome as "living foundries" to manufacture beneficial products.
    - Furthering the development of a portable system that can rapidly detect organisms and generate data on an ecosystem.
    - Prototrophy – using bacteria to transform waste to useful products or produce things we need such as vitamin D
- Consensuses
    - Inbred strains of mice should be used for experiments rather than using mice with increased genetic diversity.
    - The best approach for ISS research is the migration viewpoint, not the genetic diversity viewpoint
    - Hardware automation is preferred to citizen scientists.
    - Rather than waiting for return to Earth, data should be analyzed on the ISS so experimental parameters can be changed. Samples should also be saved.