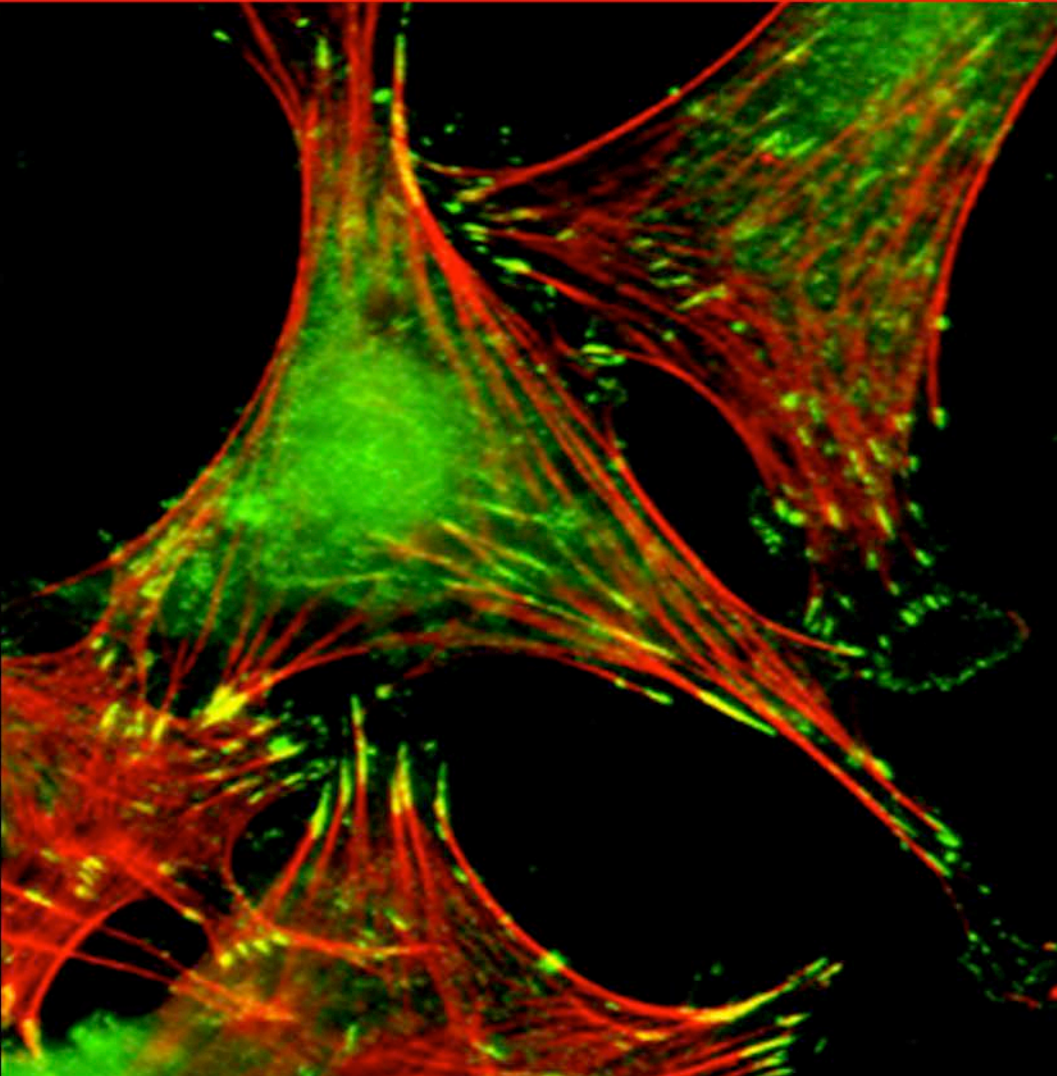




A Researcher's Guide to:

INTERNATIONAL SPACE STATION

Cellular Biology



This International Space Station (ISS) Researcher's Guide is published by the NASA ISS Program Science Office.

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a. This photo is a fluorescence microscopy image from a stem cell experiment that examined mechanotransduction in stem cell progenitors; actin fibers are red, focal adhesions are green. (Image Credit: Eduardo Almeida)

b. Back Cover: The Bioculture System flight hardware that is used to grow cell culture experiments in the ISS. (Image credit: NASA/Dominic Hart)

c. Back Cover: Cardiomyocytes in culture that will be grown in the Bioculture System hardware. (Image credit: NASA/Elizabeth Blaber)

The Lab is Open

The mission of the International Space Station Program is to advance science and technology research, expand human knowledge, inspire and educate the next generation, foster the commercial development of space, and demonstrate capabilities to enable future exploration missions beyond low-Earth orbit (LEO). This booklet, one of a series of 15 Researcher's Guides to the ISS, has been developed to provide prospective investigators with an introduction to ISS capabilities, characteristics, resources, processes, lessons learned, and knowledge gained in the general topic area of Cellular Biology.



Scientist John Freeman prepares seedlings of the model organism *Arabidopsis thaliana* for a spaceflight experiment examining changes in gene expression. (Image credit: NASA/Dominic Hart)





Unique Features of the ISS Research Environment

- 1. Microgravity**, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.
- 2. Extreme conditions** in the ISS space environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, and high-energy radiation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components.
- 3. Low-Earth orbit** at 51 degrees inclination and at a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population. This can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

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Why Use ISS as a _____ Laboratory for Cellular Biology Research: Questions and Areas

The definition of Cellular Biology used for this ISS Research Guide includes cell culture, tissue culture and related microbial (single-cell organism) experiments. These cell-based studies in microgravity support many areas of basic and applied research for space exploration and Earth applications. Such research allows the conduct of experiments with many replicates, adaptability to various mission scenarios, the ability to automate many processes, and amenability to real-time results analysis. Additionally, a number of modeled microgravity methods can be used to define and refine flight experiments thereby increasing the probability for a successful experiment in space.

A complementary research guide in this series has been developed titled *A Researchers Guide to International Space Station Microbial Research* that focuses on ISS-relevant human host-microbe/toxicology/drug interactions (Castro et al., 2013).

Research Areas

In the last 4 billion years, life has evolved and adapted in response to many physical and environmental changes on Earth. However, one key feature, the presence of gravity at its acceleration of 9.8 m/sec^2 (meter per second squared), has not changed. Thus, microgravity on the ISS that is due to the platform's continuous free-fall around Earth offers a unique opportunity for novel discoveries of cellular and tissue adaptation. These novel discoveries have applications in understanding changes to human health during long-duration spaceflight and to Earth-based medicine in the areas of biomedical research, tissue engineering, host-toxin interactions, host-pathogen interactions, vaccine development, drug sensitivity assays, and drug discovery. Using gravity as a variable enables two broad classes of space cell biology research: (a) understanding fundamental mechanisms of life's responses to changes in gravity and (b) using gravity as a tool to advance biological applications dependent on in vitro tissue culture and the emerging field of tissue engineering.



Basic cell biology experiments led by the NASA space biology and life sciences programs over the last 40 years have resulted in a substantial knowledge base of how cellular systems respond to spaceflight. Adaptations have been seen in gene expression changes, changes in cellular morphology, locomotion, trans-membrane signaling, metabolism, and cell-cell association. Advances in analytical capabilities, combined with utilizing gravity as a variable, have resulted in numerous peer-reviewed publications from spaceflight experiments. In addition, 3-D cell culture systems have been used world-wide to model microgravity and have served as valuable preflight experiment models, in-flight experiment controls, and ground-based research capabilities that have resulted in a large body of knowledge (NASA, 2010; National Research Council [U.S.], 2011; Meyers et al., 2013).

The ISS National Lab offers a valuable platform and environment for cell biology investigations, novel discoveries and innovation in a microgravity environment. Areas of opportunity include cellular biology and tissue culture studies, tissue engineering research using 3-D tissue models, biopharmaceutical production, host-microbe interactions, host-toxicology interactions, and host-drug sensitivity and resistance. For the last two opportunities, there is a strong linkage with 3-D tissue models and these questions illustrate some overlap between space cell biology and microbial research.

Research Questions

Subject Area	Potential Research Questions
Cellular Response to Microgravity and Microgravity-induced Physical Force Changes	<p>How do microgravity-induced alterations in cell shape, cell structure, function, gene expression, or other alterations contribute to the understanding of cell and tissue response to microgravity? How can this new information contribute to long-duration spaceflight and Earth-based medicine? Specific interest areas are:</p> <ul style="list-style-type: none"> • Cell and microbial replication including genetic and metabolic regulation. • Eukaryotic cell ability to generate/maintain their internal cyto-architecture. • Cell division and differentiation; cell-cell interactions and communication; intra-cellular signaling. • Gene expression/chromosome studies. • Immune cell response. • “Omics” studies. • Clarification of microgravity effects that are due to decreased mass transfer and/or physical force changes on cells. • The combined spaceflight environment effects on cells of microgravity and radiation.
Tissue Engineering of 3-D Tissue Models	<p>How can microgravity be used to improve the growth of 3-D tissue models for space-based research and Earth-based research in tissue engineering, cancer and regenerative medicine? What are the factors for increased microbial biofilm formation and the related potential for disease? What kind of ground-based radiation studies can be done using 3-D tissue models that simulate chronic spaceflight environmental conditions?</p>
Host-Microbe Interactions	<p>What is the impact of microgravity-induced alterations in the interaction of pathogens and beneficial microorganisms with cell and tissue models? How can this information be applied to operations for long-duration spaceflight and Earth-based medicine?</p>
Host-Toxicology Interactions & Host-Drug Sensitivity and Resistance	<p>What is the impact of the interaction of toxins and drugs with cell tissue models? How can that information be applied to operations for long-duration spaceflight, Earth-based medicine and commercial applications?</p>

Table 1: Opportunities for Cellular Biology Research on the ISS.


Results from Past Research

Past spaceflight experiment results indicated that mammalian cells can respond with profound shape change, altered gene expression, diminished trans-membrane signaling, modified differentiation, increased secondary metabolism, and modified tissue morphogenesis. The basis of the changes are thought to be a direct response of the cell to the change in gravity or, alternatively, to conditions in the cell culture environment created by microgravity. The former could invoke an intrinsic response structure within the cell. The latter would implicate the loss of gravity-driven convection and the hydrostatic pressure gradient, subsequent changes in mass transfer regimes, and the forced shape change as etiologic to the cell response. These alternatives pose critical questions in the microgravity sciences with impact on both biosciences and human health in space.

Knowledge of the changes in gene expression in cells facilitates the understanding of the basis of changes that occur at the tissue level, in organs, in systems, and ultimately the organism itself. This approach supports parallel investigations in human health, fundamental biology, and Earth-based biomedical and biotechnology research and development. Understanding the response of cells to microgravity can reveal underlying mechanisms in cell function that can increase fundamental knowledge as well as provide unique opportunities for biotechnology and commercial developments. The observations in tissue morphogenesis reinforce prospects for the latter.

Early studies with microbes, i.e., bacteria and fungi, showed that they reached higher population densities when grown under microgravity conditions than when cultures were grown under similar conditions on the ground. Higher cell density is likely due to a more homogeneous distribution of cells in the culture medium, unlike the settling of cells that occurs on the ground. These studies also showed that spaceflight caused some bacterial species to become more resistant to common antibiotics (Klaus and Howard, 2006). Verification of increased virulence was confirmed by ground-based studies with mice injected with space-flown bacteria (Wilson et al., 2007).


Recently, a spaceflight experiment found that space-grown cultures of the pathogen *Salmonella typhimurium* were significantly more virulent than comparable cultures grown on the ground (Wilson et al., 2007). Ribonucleic acid (RNA) microarray analyses revealed changes in the gene expression of over 160 gene transcripts, one of which was a cross-species, conserved, RNA-binding, regulator protein, Hfq,



which is involved in RNA transcription. Hfq has been found to play a role in microbial virulence of several pathogenic bacteria. These data suggested that Hfq can play a critical regulatory role in the spaceflight response of bacteria and the observed increased virulence, a result that has profound implications for long-duration spaceflight. As astronauts and cosmonauts reside for longer periods in a closed environment and use recycled water and air, there is an increased potential for microbial contamination that may impact their health, safety and performance. Adding to the concern are repeated reports that spaceflight suppresses immune function, suggesting that disease agents may be more difficult to treat during and following long spaceflights. Space-flown plants have also shown significant levels of altered gene expression (Paul et al., 2013 [Salmi and Roux, 2008]). Also, analogous to suppressed immune function in animals, alterations in cell wall composition in plants subjected to spaceflight could result in increases in the susceptibility of plants grown in space to pathogen attack (Ryba-White et al., 2001).

Spaceflight has been shown to affect a variety of mammalian cell types including bone, bone marrow cells, cartilage, and tendons, resulting in reduced matrix production or altered matrix composition (Doty et al., 1992, 1999). How spaceflight affects bone cells is not well understood. For example, how do cells sense and respond to changes in gravity? Some scientists suggest that certain cell types, when exposed to microgravity, reduce their activity or metabolism as well as the amount of new protein normally produced. This exposure to microgravity may affect the mature differentiated cell (final cell type for a specific organ-like bone) such that the cell generates a “signal” during spaceflight causing it to enter a “resting” phase. Another possibility is that the cell division cycle is delayed so that cells simply develop into their differentiated state more slowly than normal. A series of spaceflight experiments demonstrated that spaceflight suppresses hematopoietic differentiation of macrophages; other experiments showed that bone marrow cell differentiation in microgravity using murine primary macrophage cell cultures noted phenotypic shifts in the bone marrow cell sub-populations (Chapes et al., 1999).

Human renal cell cultures flown in space (Hammond et al., 1999) exhibited a genetic response to microgravity that exceeded all expectations. Based on RNA microarray analyses of spaceflight cultures compared to ground controls, more than 1,600 of 10,000 genes examined showed a change in the space-flown cells. Investigators are now beginning to focus on groups of specific genes affected by



microgravity. A NASA spinoff of this cell biology research, a rotating wall vessel (RWV) bioreactor, was developed to culture cells on the ground in an environment that models the free fall that particles undergo in cultures grown in actual microgravity. This unique bioreactor spinoff has yielded 25 patents, 19 licenses and produced thousands of systems that are now in research labs in universities, medical centers, commercial laboratories and the National Institutes of Health (Hammond and Hammond, 2001). In addition to the new cell biology knowledge, the technology and associated research findings have become part of the testing of medicines for cancers, development of transplantable tissues, and the growth of microorganisms for the development of vaccines and antibiotics.

A RWV bioreactor system (Figure 14) was successfully flown on the space shuttle (STS-70, STS-85 and STS-107) and the Russian Space Station Mir (NASA-Mir 3 and NASA-Mir 7 missions). The experiment aboard NASA-Mir 3 was a 168-day culture of bovine cartilage grown on polyglycolic acid scaffolds. Both the space-grown and ground control RWV 3-D cultures resulted in cartilage formation that was superior to the product of standard 2-D culture techniques (Freed et al., 1997). A prostate carcinoma experiment aboard Space Shuttle Columbia on STS-107 was aimed at understanding the interaction of prostate cancer cells and stromal cells and the potential of stromal cells becoming more inductive, increasing the malignant potential of the prostate cancer cells (Wang et al., 2005). Previous work performed in modeled microgravity in RWVs demonstrated that prostate carcinoma 3-D aggregates using the cell line LNCaP required the presence of prostate stromal cells to produce prostate-specific antigen (PSA). LNCaP cells grown alone in 3-D lost the ability to produce PSA, even in the presence of androgen. In contrast, LNCaP cells grown alone in 2-D produce PSA regardless of the presence of the stromal cells, suggesting the 3-D co-cultures produce a cellular architecture that's functionally more aligned with human physiology than the 3-D and 2-D monocultures (Zhou et al., 1997).

The STS-107 spaceflight was a unique opportunity to transfer experimental designs in cancer biology from ground-based modeled microgravity to actual microgravity to test whether spaceflight would more optimally model the cancer growth, differentiation and metastasis that occurs in patients (Wang et al., 2005). The initial experiment tested the hypothesis of a reciprocal cellular interaction between prostate cancer and bone stromal cells and whether permanent genotype and phenotype changes occur as a result of growth in a modeled microgravity 3-D culture condition (Wang et al., 2005). While the Space Shuttle Columbia, its crew and experiments

were tragically lost, electronic images transmitted to the ground from the onboard RWV prior to that were compared to RWV ground controls (Figure 1). Images from the flight experiment after six days of growth showed space-grown prostate organoids nearly the size of golf balls compared to 3-5 mm aggregates in the ground experiment. In previous modeled microgravity RWV-grown tissues, permanent genetic and behavior changes were found in the prostate cancer cells when co-cultured with prostate stromal cells or bone stromal cells (Rhee et al., 2001). A more recent research review suggests that controlled studies in microgravity can improve our understanding of the basic role of gravity in cancer cell growth and function and provide a novel opportunity for innovation and potentially improving treatment options in space and on Earth (Becker and Souza, 2013).

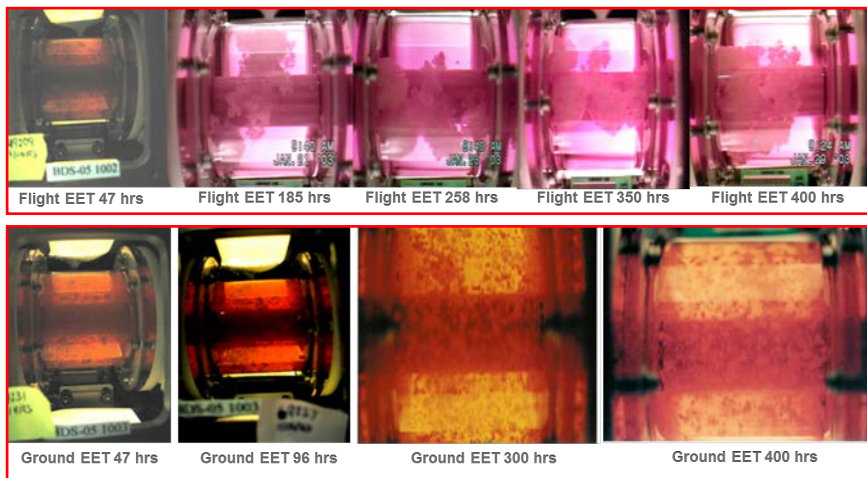


Figure 1: Images of human prostate and bone stroma cells in the JSC Bioreactor Demonstration System on STS-107 and ground control experiment: The principal investigator was L. Chung of Emory University (Wang et al., 2005). The top row is from the rotating perfused bioreactor aboard shuttle. The bottom row is from the ground-rotating, wall-perfused vessel. Experiment elapsed time in hours. Source: NASA.

A stationary incubator (Figure 4), the Biotechnology Specimen Temperature Controller (BSTC) flew on four space shuttle missions (STS-54, STS-56, STS-62 and STS-90), one NASA-Mir mission (NASA-Mir 6), and three International Space Station missions (Expeditions 3, 4, and 5). Data obtained from the experiment aboard STS-90 revealed gene expression changes in human primary renal cortical cells. The experiment included a set of ground controls: (a) a RWV, (b) a Teflon bag in a 3-g centrifuge, and (c) a static Teflon bag at 1 g, identical to that used in the BSTC in orbit. The flight cultures showed that expression of more than 1,600 out

of 10,000 genes changed, the RWV showed more than 900 genes changed, and the 3-g centrifuge showed only five genes changed, relative to the static 1-g Teflon bag (Hammond et al., 1999). Additional studies conducted using modeled microgravity in RWVs or microgravity on the shuttle or ISS are shown in Table 2.

Cell Type or Tissue Model	Response to Microgravity or Modeled Microgravity	Flight or Ground-Based System	Reference
Peripheral Blood Lymphocyte Cells (PBLs)	<ul style="list-style-type: none"> • Loss of locomotion in micro-g and modeled micro-g • Activation of PBLs with phytohemagglutinin blunted in modeled micro-g • Locomotion ability remained intact when pre-activated with anti-CD3 and IL-2 prior to culture in modeled micro-g 	STS-54 - BSTC STS-56 - BSTC Ground-Based Model - RWV	(Pellis et al., 1997)
Colon carcinoma	<ul style="list-style-type: none"> • Micro-g and modeled micro-g 3-D cultures showed lower cell proliferation, expression of EGF-R, TGF-α, TGF-β than 2-D cultures • Micro-g 3-D cultures showed significantly less apoptosis and greater carcinoembryonic antigen expression than modeled micro-g 3-D cultures 	STS-70 - RWV STS-85 - RWV	(Jessup et al., 2000)
Bovine chondrocytes	<ul style="list-style-type: none"> • Micro-g cartilage was less dense relative to ground modeled micro-g control in an RWV • Mechanical properties of micro-g-grown constructs were inferior to those of Earth-grown constructs • Implications and applications to long-duration human spaceflight, and to clinical Earth-based medicine during prolonged immobilization and reduced mechanical loading 	Mir 3 - RWV Ground-Based Model - RWV	(Freed et al., 1997)
PC-12 rat pheochromocytoma cells	<ul style="list-style-type: none"> • Long-term serial passaging of cells in micro-g • Large cellular aggregates formed in micro-g 	Mir 6 - BSTC	(Unsworth and Lelkes, 1998)

Table 2. Space Cell Biology Experiment Examples Using Actual Microgravity Culture Systems or Modeled Microgravity Systems in Rotating Wall Vessels (RWV). (Note: RWVs can be used in both flight- and ground-based studies.)

Cell Type or Tissue Model	Response to Microgravity or Modeled Microgravity	Flight or Ground-Based System	Reference
HRCE cells	<ul style="list-style-type: none"> • More than 1,632 gene expression changes in micro-g cultures • 914 gene expression changes in modeled micro-g culture • 5 gene expression changes during 3-g centrifugation • Results indicate RWV simulates some aspects of micro-g but some distinct changes in micro-g cultures not observed in modeled microgravity 	STS-90 - BSTC Ground-Based Model - RWV	(Hammond et al., 1999)
Ovarian carcinoma cells	Antigenic protein can be extracted from RNAlater™-fixed samples stored in less than optimal conditions on ISS	ISS Expedition 3 - BSTC	(Hammond et al., 2005)
Lymphoid tissue	<ul style="list-style-type: none"> • Human lymphoid cultures in micro-g did not respond to antigenic or polyclonal challenge unless stimulated prior to exposure • Human tissues and cells in modeled micro-g RWV cultures did not respond to recall antigen or polyclonal activator challenge unless stimulated prior to culture 	ISS Expedition 4 - BSTC Ground-Based Model - RWV	(Fitzgerald et al., 2009)
HRCE cells	Quantitative proteomic information can be acquired from samples stored in less than optimal conditions on ISS	ISS Expedition 4 - BSTC	(Hammond et al., 2006)
Host-microbe	<ul style="list-style-type: none"> • Increased virulence and stress resistance of modeled micro-g RWV-grown <i>Salmonella enterica</i> Serovar Typhimurium • Increased virulence and unique gene expression and regulation of <i>S. Typhimurium</i> cultured during spaceflight • Reproduced finding of increased virulence of <i>S. Typhimurium</i> cultured during spaceflight and identified the impact of media content on this change • Increased virulence in micro-g paralleled RWV-grown cultures and validated use of RWV as a micro-g analog 	STS-115 STS-123 Ground-Based Model - RWV	(Nickerson et al., 2000) (Wilson et al., 2007) (Wilson et al., 2008)
Review article	Tissue engineering in micro-g bioreactors relevant to space biology and medicine		(Barzegari and Saei, 2012)
Human epithelial cells	Gene expression from cultures in orbit, before or after infected with <i>S. Typhimurium</i>	CCM STS-131	Analysis continuing
Mouse embryonic stem cells	<ul style="list-style-type: none"> • Tissue regenerative potential of stem cells decreased during micro-g • Inhibited cell differentiation seen • Gene expression analysis/bio studies ongoing 	CCM STS-131	Analysis continuing

Opportunities for Research on the ISS: NASA and Center for the Advancement of Science in Space (CASIS)

In 2005, the U.S. Destiny module of the ISS was designated the ISS National Lab (ISSNL) and is the in-orbit research laboratory primarily used by NASA-sponsored investigators but also increasingly by other federal entities and the private sector. In 2011, NASA chose the non-profit CASIS to be the sole manager of the ISSNL for non-NASA-funded research. Their mission is to maximize its innovative use to pursue national priorities in science, technology, engineering and mathematics and to expand the U.S. economy.

NASA

NASA's selection of research projects is guided by recommendations of the National Research Council's 2011 Decadal Survey Report, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" (National Research Council [NRC; U.S.], 2011). The NASA-developed "Fundamental Space Biology (FSB) Science Plan" provides an implementation strategy and roadmap based on NRC recommendations and available flight and fiscal resources (NASA, 2010). Extractions of research subject areas and research questions from both of the above sources are included in Section 2, Table 1. And content in Section 3, regarding results from past research, was extracted in part from the FSB Science Plan for Cell, Microbial and Molecular Biology. Information on funding sources for this research is provided below in the section on solicitations, proposals and funding.

CASIS

Another option for flying an experiment is through the Center for the Advancement of Science in Space (<http://www.iss-casis.org>). CASIS is a nonprofit organization tasked by the U.S. Congress and NASA with promoting and enabling non-NASA research on ISS. CASIS can support all stages of payload development (<http://www.iss-casis.org/CASISBasics/ForResearchers/SupportServices.aspx>) and can match principal investigators with Implementation Partners (<http://www.iss-casis.org/CASISBasics/ForResearchers/DirectoryofImplementationPartners.aspx>) who can provide heritage hardware, new flight instrumentation or help to accommodate PI-provided payloads. Implementation Partners can collaborate with

NASA to help streamline the process of payload development, testing, transport to orbit and integration with the ISSNL.

Solicitations, Proposals & Funding

NASA Research Announcements are managed through the online NASA Solicitation and Proposal Integrated Review and Evaluation System (NSPIRES), <http://nspires.nasaprs.com/external/index.do>. This system supports the NASA research process from the release of solicitation announcements through the peer review and selection processes.

Additional research announcements can be found at the Center for the Advancement of Science in Space, <http://www.iss-casis.org/Home.aspx>. CASIS also encourages unsolicited proposals and sponsors prizes.



Proposals submitted for NASA's research opportunities undergo scientific and technical review. Center for the Advancement of Science in Space has a similar process that is streamlined to optimize ease of user access to the International Space Station National Lab.

A detailed catalog of previous, current, and proposed experiments, facilities, and results, including investigator information, research summaries, operations, hardware information, and related publications is available at www.nasa.gov/iss-science through the NASA ISS Program Office. Additionally, details pertaining to research previously supported by the Space Life and Physical Sciences Research and Applications Division of NASA's Human Exploration and Operations Mission Directorate can be located in their Division "Task Book" in a searchable online database format at: <https://taskbook.nasaprs.com/Publication/welcome.cfm>.

Funding sources for research on the ISSNL are available via NASA, CASIS, other Federal entities and self-funding by the private sector. NASA will continue to cover the cost of onboard operation and maintenance of research payloads and their transport to and from the ISS.

Facilities for Cellular Biology Experiments on the ISS and How to Choose Them

In general, U.S.-developed ISS facilities fall into three availability categories: currently aboard; in development with a targeted launch date; and certified/flown on the STS and undergoing re-certification for ISS. NASA can provide access to hardware in these categories. A fourth category is facilities or equipment available from a commercial source either through NASA, through CASIS or provided directly by the developer. Some available and soon-to-be available U.S. facilities that support cellular biology research are listed below. A supplemental list with both U.S. and international partner facilities is included in the Appendix.

Advanced Biological Research System (ABRS)

The ABRS is a single-locker system providing two growth chambers. Each growth chamber is a closed system capable of independently controlling temperature, illumination and atmospheric composition to grow a variety of organisms including microorganisms, plants and small arthropods (insects and spiders).



Figure 2: The Advanced Biological Research System unit in an International Space Station Expedite the Processing of Experiments to Space Station rack at NASA's Kennedy Space Center. Image courtesy of the Bionetics Corporation.

BioCulture System (formerly Cell Culture Module)

The BioCulture System is still in development for NASA by Tissue Genesis, Inc. (<http://tissuegenesis.com>) and its functions are profiled in Lessons Learned as an example of how STS hardware can be upgraded and additionally automated for use on the ISS for longer-duration and sequential experiments. The Cell Culture Module shown in Figure 3 was an STS-era system utilized for many cellular biology studies (see Table 2). The BioCulture System will be an automated and accessible cell culture and experiment system that provides controlled maintenance and growth in microgravity. The containment system allows for initiation, intervention, and analysis of ongoing experiments on ISS. The primary component is a hollow fiber bioreactor that is designed to deliver nutrients and remove waste via multiple, tightly packed perfusion fibers. The hollow fiber system is particularly suited for microgravity cell culture where nutrient transfer is limited to diffusion. Examples of previously flown cells include myoblasts, osteoblasts, fibroblasts, endothelial cells, stem cells, muscle cells, and bone cells. Standard laboratory processes such as media feeds, waste removal, sample collection, protocol additions, or other additions are automated. The system is also designed for glovebox-assisted crew access to the removable and disposable fluid compartments, allowing for in-orbit culture initiation from frozen stores, and recovery of cells/samples for storage and transport in low-temperature freezer systems. The system is reconfigurable and customizable.

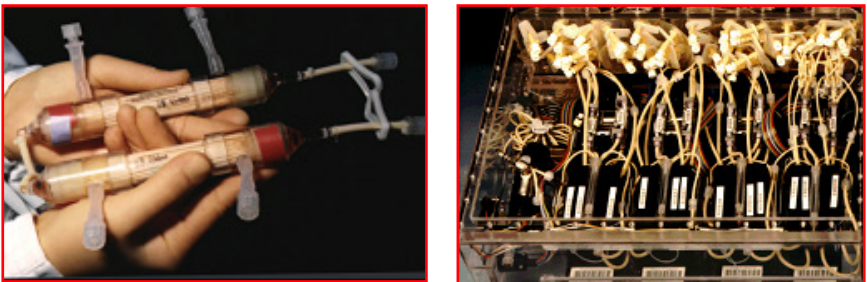


Figure 3: The Cell Culture Module with Multiple Bioreactor Units.

Biotechnology Specimen Temperature Controller (BSTC)

The BSTC is a static tissue culture incubator designed to allow multiple short-term experiments to be controlled simultaneously. It is a single chamber that holds 32 Teflon bags of 15-30 ml each. It can accommodate OptiCells™ (<http://>



Figure 4: The Biotechnology Specimen Temperature Controller.

www.the-scientist.com/?articles.view/articleNo/13305/title/Tissue-Cultural-Revolution/) and provides a selectable thermal environment of +4 to +50°C. Air/CO₂ mixtures can be provided via an external Gas Supply Module (GSM). Media exchanges and sampling for off-line metabolic analysis, cell sampling, and 3-D tissue aggregates for fixation is performed manually. The BSTC provides TCP/IP Ethernet connection for communication with the Expedite the Processing of Experiments to the Space Station (EXPRESS) Rack interface

computer.

Commercial Generic Bioprocessing Apparatus (CGBA)

The CGBA provides programmable, accurate temperature control—from cold storage to a customizable incubator—for experiments that examine the biophysical and biochemical actions of microorganisms in microgravity. CGBA can be used in a wide variety of biological studies, such as antibiotic-producing bacteria and cell culture studies. It is designed to be installed in the EXPRESS Racks for operation on ISS.

The CGBA can be fitted with a number of bioprocessing inserts including:

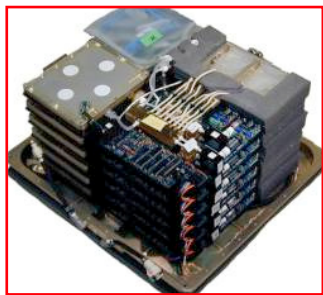


Figure 5: Multiple Orbital Bioreactor with Instrumentation and Automated Sampling.

- **Multiple Orbital Bioreactor with Instrumentation and Automated Sampling (MOBIAS):** MOBIAS consists of stackable trays, each of which provides an appropriately controlled, sterile sample-processing environment with passive gas exchange, automated sampling, and waste removal. Each tray contains its own array of sample, cultures, medias, waste bags, and connectors.

- The Gas Exchange - Group Activation Packs (GE-GAPs):** GE-GAPs have an aluminum shell that enables heat transfer and a gas-permeable membrane that covers the openings in the wall to allow passive gas exchange for the experimental samples located in the GAPs inside BioServe's fluid processing apparatus, a piece of hardware similar to a small test tube that can activate, mix, grow, and terminate biological experiments.

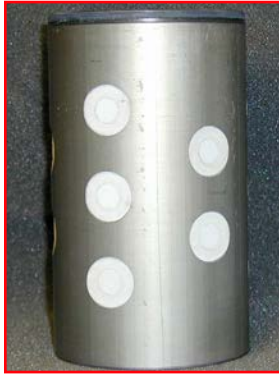


Figure 6. OptiGAP (left) and Gas Exchange - Group Activation Packs (right).



Figure 7. BioServe Culture Apparatus.

- BioServe Culture Apparatus (BCA):** The BCA was developed for biological studies, including suspension cell culture and tissue engineering studies. The BCA allows for passive gas exchange in a sterile environment and can provide growth media, sampling and fixation of cultures. The BCA allows for thermally controlled or software controlled time-course sampling.
- Culture Habitat (CHab):** The CHab consists of six culture chambers, which may be inoculated sequentially or concurrently. It provides sample fixation or activation, passive gas exchange, environmental monitoring sensors, video and still imagery, and automated, configurable experiment control. The CHab can be used for research with cell cultures, microorganisms and viruses.

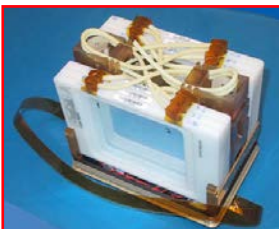


Figure 8. Culture Habitat.

Multipurpose Facilities

- **The Microgravity Science Glovebox (MSG):** The MSG was built by the European Space Agency (ESA) but is operated by NASA and is the largest glovebox flown in space. It is located in the U.S. Destiny module and provides containment for experiments, insuring that small or hazardous materials do not escape and float about in the cabin. Crew members utilize one or more of four gloveports to manipulate materials or experiment systems that are transferred inside via a special opening. It has a video system and data downlink to allow control of enclosed experiments from the ground.



Figure 9. Microgravity Science Glovebox.

- **Other:** Various facilities are available on the ISS relevant to cellular biology research including: image processing and downlink systems; microscopes; biosample freezers; biosample collection, handling, fixation and storage systems; and a portable glovebox. More information on biological research facilities and multipurpose facilities is available in the NASA publication “International Space Station Facilities: Research in Space 2013 and Beyond,” accessible online at www.nasa.gov/pdf/739318main_ISS%20Utilization%20Brochure%202012%20Screenres%203-8-13.pdf.

Lessons Learned

ISS Payload/Experiment Logistics & Operations Scenarios

Discussions of experiment design with knowledgeable implementation partners, ISS personnel, and hardware providers are important for the planning of experiments and preparation of grant proposals. Additional information and points of contact are provided below. Some basic guidelines include:

- Standard laboratory procedures such as pipetting for fluid transfers must be modified for containment purposes. Many payloads use syringes with cannula attachments interfaced with needle septa.
- Scientists requiring cold stowage should define their requirements to ensure proper storage of reagents, cell culture stocks, and samples for durations that will prevent the loss of viability. Selection of fixatives, chemicals, and other reagents are assigned hazard classifications that determine the required levels of containment.
- The current path for experiment payloads to reach the ISS is by transfer (turnover) to a SpaceX Dragon spacecraft at NASA's Kennedy Space Center between L-24 and L-18 hours before launch. The procedure must allow for enough time from launch to docking to transfer to the ISS experiment integration site when designing an experiment.
- Dragon is the only transportation capsule that currently returns payloads. The need for live biosample return must be carefully considered based on the duration of time the live specimens will be in a 1-g environment before turnover back to the PI.
- There is limited cold stowage and ambient stowage availability for ascent to and descent from the ISS. The General Laboratory Active Cryogenic ISS Experiment Refrigerator freezer has a permanent rack location assigned to it within Dragon.
- Overall: The Dragon capsule does not include crew, so manual experiment procedures need to be deferred until experiment integration occurs within the ISS. Return of biosamples to the PI after re-entry takes approximately 72 hours.

Implementing Optimization Strategies for ISS Operations: The BioCulture System



Figure 10: The BioCulture System with ten independent, removable cassettes.

The BioCulture System (Figures 10-13) is in development for the ISS. It is under construction by Tissue Genesis, Inc. who developed the earlier Cell Culture Module flown several times on the Space Transportation System (STS). The STS launched to LEO, stayed in orbit for about two weeks and then re-entered and landed on a long airport runway. STS experiments and research specimens were loaded pre-launch, transported to orbit and could be activated by onboard crew within a few hours. The ISS provides a new opportunity to do extended research in a true laboratory environment on

its semi-permanent orbital platform, but the new payloads and supply replacements need to be separately transported there from the ground. Increased research facility and experiment automation, as designed for the BioCulture System, will provide significant benefits because of the already significant requirements on crew time for supporting ISS logistics and maintenance operations as well as the extended experiment operations.

The BioCulture System provides crew access to cellular biology research on station through the use of cassette containment that can be opened and then resealed in the Microgravity Science Glovebox facility (Figure 9). This access allows for culture initiation, replenishment, recovery and improved sample collection and storage. The automated experiment functions have been increased and improved including forward and reverse perfusion, injections with paired fraction collections, cross membrane fixation and additional fluidic control points that increase the number of automation options. The media, additives and samples are stored in the individual cassette refrigerator compartment to lessen the number of manual operations. This feature preserves the media quality and improves the storage of samples collected. Additionally, the BioCulture System provides a full range of temperature control supporting conventional cell culture models and enables research with bacteria and embryos. The individual experiment elements required for execution (pump, bioreactor, diverter valves, oxygenator, pre-heat, media reservoir, sample, injection and sump) are packaged as a disposable insert. When an experiment is completed,

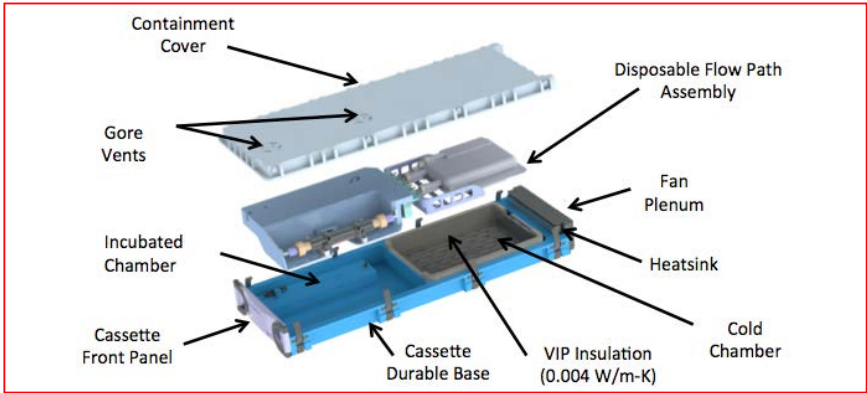


Figure 11: Cassette Assembly.

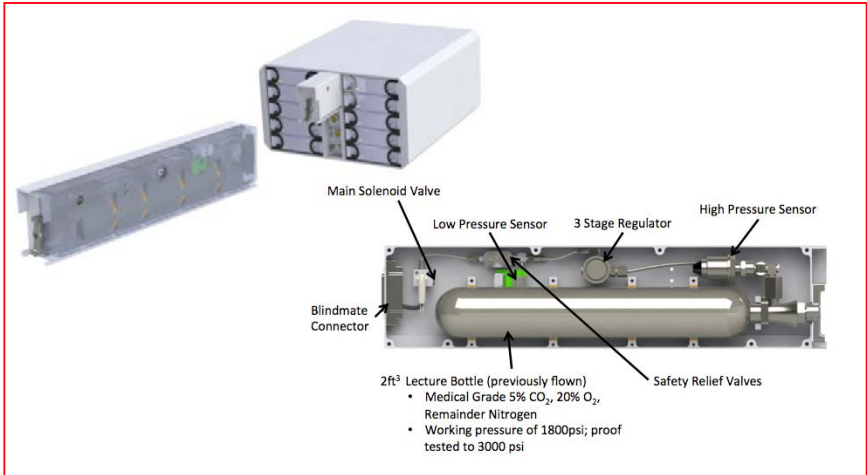


Figure 12: Removable Gas Supply Assembly.



Figure 13: A Single bioreactor unit removed from a bioculture system cassette.


the one-piece disposable insert is removed and a new disposable insert is added to start a new experiment within the BioCulture System. The experiment parameters are displayed on an experiment specific secure digital card that travels with the disposable insert.

The Rotating Wall Vessel (RWV) Bioreactor: A Microgravity Model System

Although microgravity cannot be created on Earth, some aspects can be effectively investigated using a modeled (or analog) microgravity cell culture system previously designated the RWV bioreactor. It has been used as a partial model for microgravity in many cell culture and tissue engineering studies. Four key principles make up the RWV bioreactor design: (a) low hydrodynamic shear, (b) co-location of particles with differing sedimentation rates, (c) 3-D spatial freedom, and (d) cells are continuously falling at terminal velocity through the culture fluid and not sedimenting on a surface. These features provide an environment that is similar to some aspects of microgravity, facilitating the development of 3-D tissue models that produce the cytoarchitecture and functional markers of in vivo tissues.



Figure 14: Examples of the rotating-wall vessel bioreactor systems available through Synthecon, Inc., Houston, Texas. Disc-shaped cylindrical vessels on the left are 10-ml and 50-ml high-aspect rotating vessels (HARVs) attached to a four-place rotator base (horizontal-axis rotation). The cylindrical vessels on the right are 55-ml, 110-ml, and 250-ml slow-turning, lateral vessels on top of the power supply positioned as controls (vertical-axis rotation) for the modeled microgravity produced by the HARVs on the left. Source: Synthecon, Inc. (<http://www.synthecon.com/rotary-cell-culture-systems.html>).



The RWV has been extensively characterized and used for a wide variety of cell and tissue types since the original invention by NASA in the mid to late 1980s (Barrila et al., 2010; Barzegari and Saei, 2012; Becker and Souza, 2013; Duray et al., 1997; Unsworth and Lelkes, 1998). Details of hardware designs for two different incubator-based RWV systems, along with operating procedures were previously described (Becker et al., 1993; Schwarz et al., 1992). A slow-turning, lateral vessel (STLV) consists of a cylindrical vessel with a concentric silicone membrane oxygenator and a high-aspect rotating vessel (HARV) that is a variation of the cylindrical design with the silicone membrane oxygenator on one end of the cylinder (Figure 14). The reduced cross-sectional thickness of the HARV facilitates gas exchange, thereby providing an improved environment for cells and tissue types that have higher metabolic requirements. With the vessels completely filled with culture media (zero headspace), the media viscously accelerates until both the wall and fluid mass are rotating at the same angular rate. This eliminates destructive shear gradients associated with the boundary layer and wall effects inherent in conventional bioreactors (Schwarz et al., 1992). Cells obey simple kinematics and are uniformly suspended in the fluid. Solid-body rotation reduces the shear and turbulence normally associated with impeller stirred bioreactors, thereby simulating some aspects of microgravity (Dedolph and Dipert, 1971; Tsao et al., 1992). The vessel operates with no internal moving parts at an unusually low shear regimen of 0.2 dyne/cm² (dyne per square centimeter).

Developing and Flying Research on the ISS

Conducting Space Research: The ISS Environmental Conditions

Understanding key aspects of the ISS research environment is critical to appropriate experiment design. Three important elements of the internal environment are profiled below.

Microgravity

Microgravity on the ISS is typically about one millionth of Earth gravity and is effectively an environment of near-weightlessness. But gravitational force is not the only one that can be relevant to a biological experiment (DeLombard et al., 2004). Disturbances may be caused by vibrations (e.g., pumps, fans, exercise systems) and/or transient operational phenomena (e.g., valve operation). The magnitude of these impacts on the gravitational environment can range from 0.01 g (briefly for a thruster jet) to below one millionth of 1 g (prolonged for atmospheric drag in orbit).

For biological experiments, it is important to understand the differences between the direct effects of microgravity in which a living system perceives changes in the gravitational force directly, and indirect effects in which the system responds not to the lack of gravitational force itself, but to changes in the local environment induced by the conditions of microgravity. The reduction in gravitational forces on biosystems results in decreased buoyancy-driven flows, rates of sedimentation and hydrostatic pressure. In general, fluid dynamics are also altered, and there is a near absence of convection (National Research Council [U.S.], 2011).

Radiation Exposure

NASA's current life sciences goals are focused predominantly on understanding the effects of space radiation on humans in space and developing strategies to mitigate adverse effects. While there is a large body of existing literature on the effects of low linear energy transfer (LET) radiation such as gamma rays and X-rays on biological samples, including data from long-term animal studies, clinical studies and others, the information on radiation of the kind encountered in space (e.g., protons and high-LET radiation such as heavy charged ions) is less well-defined (National Research Council [U.S.], 2011).

Crews aboard the space station receive an average of 80 mSv (millisievert, measurement for radiation dose) for a six-month stay at solar maximum (the time period with the maximum number of sunspots and a maximum solar magnetic

field to deflect the particles) and an average of 160 mSv for a six-month stay at solar minimum (the opposite condition). Although the type of radiation is different, one mSv of space radiation is approximately equivalent to receiving three chest X-rays. On Earth, we receive an average of 2 mSv every year from background radiation alone.

Ambient Gas Concentrations and Pressure

The air within the ISS is dynamically controlled to be close to the gas concentrations and total pressure to our atmosphere on Earth. Nitrogen and oxygen are stored in tanks and released automatically based on sensor readings, and carbon dioxide is chemically absorbed.

Experiment Accommodation on the ISS

Experiment payloads are all held within International Standard Payload Racks (ISPRs) within the ISS. Each ISPR consists of an outer shell that provides a set of standard interfaces, a support structure and modular equipment for supporting research hardware. Each can accommodate one or several experiments.

Through the ISPRs, the ISS payload experiments can be provided with the following ISS resources available on the U.S. laboratory, also known as Destiny:

- Electrical power
- Thermal control
- Command/data/video
- Vacuum exhaust/waste gas
- Gaseous nitrogen

The Expedite the Processing of Experiments to the Space Station (EXPRESS) Rack System is available to support small, sub-rack payloads with power, data and cooling within an ISPR. EXPRESS racks were designed to accommodate payloads originally fitted to shuttle middeck lockers and International Sub-rack Interface Standard drawers, allowing previously flown payloads to easily transition to flight on the ISS (National Research Council [U.S.], 2011).

Developing Your Space Flight Experiment

Several milestones along your experiment development path are described below as a NASA-supported process that applies to PIs who are NASA-funded grantees (see Section 4 - Opportunities for Research on the ISS, above). However, a similar, streamlined process is required for PIs who are not NASA-funded including CASIS grantees, other government grantees or self-funded commercial entities. In this non-NASA funded case, PIs are considered participants on the CASIS-managed ISSNL, and support is provided to them by CASIS and one or more Implementation Partners (<http://www.iss-casis.org/CASISBasics/ForResearchers/DirectoryofImplementationPartners.aspx>).

When a proposal is accepted as a spaceflight candidate after undergoing scientific and technical review, a flight experiment team is formed, the development cycle is initiated, then proceeds in phases. The PI will be supported by an assigned project scientist who functions as the advocate and liaison for the PI and assists with the experiment development process.

For optimal use of the limited in-orbit resources, experiments may be combined where feasible—for example, those requiring similar biospecimens and hardware. Such teams will work together to achieve individual objectives within the bounds of constrained resources. These teams will be manifested and implemented as a group.

Principal Investigator (PI) Role and Responsibilities

The fundamental role and responsibilities of the experiment PI are:

- Definition of the basic scientific and operational requirements for the experiment.
- Working with the project team to ensure that research objectives are maintained during design, development and flight.
- After the experiment has been completed, the PI is required to complete and submit the analyzed data and a final report to NASA and to publish the results, as appropriate.
- Compliance with all safety training, policies and procedures as required by NASA.

Implementation Team Role and Responsibilities

The spaceflight Implementation Team is led by a NASA-provided payload or project manager. The team works together to manage and implement the phases of the experiment development cycle. The NASA-provided project scientist will work directly with the PI throughout this process.

Definition, Documentation and Testing of a Space Flight Experiment

The following is representative of the documentation, testing and information that will be required.

To complete a successful experiment in microgravity, a detailed analysis and definition of the proposed experiment must be done. All requirements for the execution of the experiment must be identified and described and a feasibility analysis conducted.

Additionally, the required hardware and resource requirements must be identified in detail for all phases: preflight, flight, and postflight, including assessment of the maturity of experiment and financial resources required for experiment. If needed, the design, development and manufacture of experiment-unique hardware will be conducted, and experiment hardware interfaces and operations will be verified through testing.

The PI will work with the assigned Project Scientist to complete all of the required phases for development and flight readiness. A series of reviews of the experiment will be conducted, including reviews for safety requirements.

As per NASA life sciences flight experiment management policy, if satisfactory results are not obtained during testing, a flight experiment may be deselected and perhaps considered for ground research based on peer review; or it may need to be cancelled altogether.

Development of Spaceflight Experiment Requirements

The foundation of the spaceflight experiment is the clear definition and identification of all aspects of the proposed experiment. It is critical that the PI work with the project team in a series of activities that are necessary for developing the spaceflight experiment.

Science Ground Testing

The project scientist and the flight experiment team will support the PI in defining the types of testing that will be required before flight in order to mitigate risks and increase the chance of a successful experiment. These tests would include such things as validation of new hardware and practice runs to optimize and streamline in-orbit operations. The test results will define requirements, procedures, hardware setting and configurations.

Ground Controls

Proper ground control experiments are essential for successful and scientifically sound spaceflight experiments. Conducting ground control experiments:

- Synchronous ground control experiments (housed in flight-type hardware on the ground) will be supported at NASA.
- If appropriate, ground control experiments may be performed on a time delay to allow for provision of temperature and other parameters that closely simulate actual flight conditions.
- Ground control experiments will be supported by NASA.

Hardware Biocompatibility Tests


Hardware biocompatibility testing is warranted in some cases to ensure that biosamples and/or organisms do not encounter unexpected difficulties while housed in payload system hardware under the prescribed experimental conditions.

Project Integrated Tests

Integrated testing of expected hardware operations and procedures on the ground may be warranted. The appropriate testing will be identified by the experiment team in collaboration with the PI.

Conducting a Spaceflight Experiment: Payload Flight Operations

Launching and delivering a life sciences experiment to the ISS requires extensive preparation in support of the logistics activities. NASA provides laboratories for preflight preparation and postflight experiment activities. All of the laboratory



equipment that is needed for pre and postflight experiment processing must be identified, and specialized equipment may have to be provided by the PI. The assigned NASA project scientist will assist and work directly with the PI to provide the necessary information and documentation for flight logistics and operations. Contingency planning for launch delays is also part of logistics planning.

Typical preflight activities include launch site facility trial runs for preflight experiment preparation and processing, training of astronauts for in-flight experiment operations and activities supporting transfer of experiment payload elements to the launch area. Additionally, trial runs for postflight experiment activities to be conducted in laboratories on the ground upon return of the experiment to Earth must be considered.

Funding Opportunities/ _____ Points of Contact

NASA research announcements are managed through the NASA Solicitation and Proposal Integrated Review and Evaluation System. This Web-based system supports NASA research from the release of solicitation announcements through the peer review and selection processes (<http://nspires.nasaprs.com/external/index.do>).

Additional research announcements can be found at the CASIS website:
<http://www.iss-casis.org/Home.aspx>.

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Appendices

Supplemental Hardware List

This list, provided for general information purposes, includes both U.S. and international hardware, arranged by type (stand-alone incubator systems with an associated centrifuge; stand-alone incubator systems with no centrifuge; and tissue culture systems requiring the ISS incubator). Cellular biology research facilities potentially available for ISS fall into three general categories: currently certified for ISS (www.nasa.gov/pdf/739318main_ISS%20Utilization%20Brochure%202012%20Screenres%203-8-13.pdf); in development for ISS; and certified/ flown on STS with potential for re-certification for ISS. This information is meant to describe some top-level hardware capabilities and status for each system.

NASA approval is needed in order to utilize any non-NASA flight hardware listed below since access to such items is often based on international agreements and hardware utilization exchanges.

Stand-alone Incubator System with an Associated Centrifuge

Cell Biology Experiment Facility (CBEF; Saibo; JAXA)

o Incubator

- Temperature settings: +15°C to +40°C (in 0.1°C increments).
- CO₂ Settings: 0 to 10 percent (in 0.1-percent increments).
- Humidity settings: 20 to 80 percent (in 1-percent increments; dependent on the humidity outside of the CBEF).
- Quantity of Biological Experiment Units (BEUs): six; capable of canister heating.
- Provides power, sensor data, commanding, and analog video.

o Centrifuge

- Same environmental system as the Incubator.
- Gravity: 0.1 g to 2 g at 112.5-mm radius point; 20 rpm to 140 rpm in 1 rpm steps
- Quantity of BEUs: four; no canister heating capability.
- Provides power, sensor data, commanding, and analog video.

o **User Interface for a BEU**

- Power DC +5 V, -5 V, +12 V, -12 V: one line each.
- Sensor Output 0 V to 5 V.
- Command 1 bit: two lines.
- Standard JEM Video Specifications.
- Shield GND.

o **BEU for CBEF Utilization**

- Shell for housing User-developed science equipment.
- Standard user interfaces to the incubator and centrifuge.
- 210 mm by 80 mm by 130 mm (L X W X H).
- Installed with the 80 mm by 130 mm face connected to the incubator or centrifuge turntable.
- Centrifugal force vector is parallel to the 210-mm length.

o **CEU**

- 10 cm² (3 ml) and 30 cm² (9 ml) culture chambers.
- Two chambers per CEU.
- Supports adherent tissue culture cell lines.
- 210 mm by 80 mm by 130 mm.
- Medium circulation pump for the sterile, closed fluid loop.
- Fresh Medium Container (25 ml minimum to 80 ml maximum).
- Spent Medium Container.
- Temperature and Humidity Sensors.
- 10-day mission duration, cell processing is postflight only.

o **CEU2**

- 3 mm by 3 mm by 60 mm culture chambers.
- Two chambers per CEU, fully independent
- Supports non-adherent tissue culture cell lines.
- 290 mm by 80 mm by 130 mm.

- Medium circulation pump for fluid loops; flow valves.
- Fresh Medium Container (50 ml) and Spent Medium Container.
- Temperature and humidity sensors.
- Automated sampling/fixation (1.5 ml; nine bags per chamber); sampling/fixation system is detachable for stowage.
- CCD camera with illumination laser, video data downlink.

Biolab (ESA)

- o The Incubator houses two centrifuges; all of the Experiment Cassettes (EC) are installed on one or both of the centrifuges.

o Incubator Chamber

- Temperature settings: +18°C to +40°C.
- CO₂ settings: 0 to 0.2 percent (in 0.01-percent increments); 2 to 5.5 percent (in 0.1-percent increments).
- O₂ settings: 15 to 22 percent (in 1-percent increments).
- Relative Humidity Settings: 60 to 90 percent (in 1-percent increments).

o Centrifuge

- Two independently controlled centrifuges.
- Gravity settings: 0.001 g to 2 g; Radial acceleration at center of EC can be selected in 40 steps.
- Six EC per centrifuge.
- Provides power, data, sensor data, video.
- Lighting and video system.
- Provides a liquid handling mechanism for delivering and withdrawing fluids.

o EC for the Biolab Incubator

- Shell for user-developed experiment hardware.
- Provides interfaces to the Biolab life support system, power, data, external light, fluid handling mechanism, etc.
- Clear cover for use with the external lighting and video system.

- Available volumes: 60 mm by 60 mm by 100 mm.
- Six ECs per rotor.
- In development: Advanced EC-2.5 liter volume and video connections; two per rotor.

Kubik (ESA)

- o Two versions: Amber (centrifuge insert) and Topaz (no centrifuge).
- o External dimension: 366 mm by 366 mm by 366 mm.
- o Internal dimension: 260 mm by 260 mm by 138 mm.
- o Amber holds 16 standard Type 1 containers (static) and eight standard Type 1 containers on the centrifuge.
- o Topaz holds up to 40 standard Type 1 containers, all static.
- o Temperature settings: +6°C to +38°C (in increments of 1°C).
- o Gravity settings: 0.2 g to 2 g (in increments of 0.1 g).
- o No atmospheric control.
- o No power connectors.
- o No data, commanding, or video interfaces.
- o Flown on Soyuz and ISS in the Russian Module.

Stand-alone Incubator System with No Centrifuge

Cell Culture Module (CCM; WRAIR; TGI) - Now Evolving to the ISS BioCulture System

- o Hollow Fiber Bioreactors (max of 24 [3] ml or max of 16 [7] ml bioreactors); Custom bioreactor accommodated, mix types in CCM.
- o Supports adherent cells, non-adherent, and 3-D tissue culture.
- o Temperature Settings: +4°C to +39°C.
- o Humidity: Ambient in Module.
- o CO₂ control: 5 percent.
- o Fluid loops and oxygenator system for CO₂ gas exchange perfusion system.
- o Fluid/Medium delivery rate can be user-defined.

- o User defined medium bags, fixative bags, and sampling bags need.
- o Sensor (temp, CO₂) data collection and downlink capable.
- o Automated sampling and injection.
- o Flown on shuttle (18 flights), must be ISS-certified, CCM-A, B, and C.

Rotating Wall Perfusion System (RWPS; NASA JSC)

- o 125-ml rotating wall reactor vessel; one vessel.
- o 3-D cell/tissue culture.
- o Continuous gas perfusion system; automated medium circulation.
- o Three 2-liter fresh medium bags and three 2-liter spent medium bags; medium stowage tray holds seventeen 1-liter concentrated medium bags, which are diluted prior to use.
- o Controllable rotation rate.
- o Supports 3-D cultures up to 120 days.
- o Temperature setting: +36°C.
- o Humidity: passive.
- o CO₂ control: uses the accessory GSM.
- o Manual sampling.
- o No previous flights; requires ISS flight certification.

Advanced Separations (ADSEP; TechSHOT)

- o CellCult system: 50 ml culture container, perfusion system, fluid loops, automated sampling and injection; three CellCult Cassettes per incubator.
- o Supports non-adherent.
- o Temperature settings: +4°C to +39°C (independent for each cassette).
- o Humidity: ambient in module.
- o CO₂ control: ambient in module.
- o Flown on shuttle only, requires ISS-certification.

EDU-1R (NASA JSC)

- o 125 ml rotating wall reactor vessel; one vessel.

- o Non-adherent cultures; adherent cultures on microcarrier beads.
- o Gas perfusion system; automated medium circulation.
- o Supports cultures up to 20 days (gas cylinder volume dependent).
- o Pre-programmed medium feeding schedule.
- o Differential rotation of the inner and outer cylinders; controllable rotation rate.
- o Temperature setting: +30°C to +45°C.
- o Humidity: passive.
- o CO₂ control: see the accessory GSM.
- o Manual sampling.
- o Previously flown on ISS; requires ISS-certification.

Automated Rotating Culture System (ARCS; NASA JSC)

- o Rotating wall reactor vessel; one vessel.
- o Supports cultures up to 20 days (internal system) or longer (GSM).
- o Intermediate to large scale 3-D cultures and cell aggregates.
- o Gas perfusion system, automated medium circulation.
- o Pre-programmed medium feeding schedule.
- o Medium aeration, medium infusion, and reactor vessel imaging.
- o Temperature setting: +30°C to +45°C.
- o Humidity: passive.
- o CO₂ control: internal system and accessory GSM.
- o pH monitoring and control.
- o Manual sampling.
- o Prototype.

Tissue Culture Systems Requiring ISS Incubator

Multiple Orbital Bioreactor with Instrumentation and Automated Sampling (MOBIAS; BioServe)

- o Gas-permeable bags.
- o Supports non-adherent cells and 3-D tissue culture.
- o Two temperature zones: +10°C to +37°C to +4°C to +37°C.
- o Humidity: ambient in module.
- o CO₂ control: ambient in module.
- o Stacked trays; each tray with medium and waste bag, fluid circulation loops, incubation zone, cold/incubation zone, up to 50 ml culture volume (one bag or multiple bags), video card, motor drive card, fully automated.
- o Incubator required for power.
- o Previous shuttle flights, BioServe has indicated it is ISS-certifying all of its flight hardware; no crew access to incubation chamber.

Single Loop Cell Culture (BioServe)

- o Adherent and non-adherent.
- o 10-ml cell culture volume.
- o Continuous perfusion of medium for gas exchange.
- o Six 3-ml containers for inoculums or samples.
- o Automated inoculation or sampling per a pre-set time sequence.
- o Fixatives pre-loaded in sampling containers.
- o Fresh medium bag waste bag.
- o Provides temperature and humidity data.
- o Requires power and thermal conditioning from an incubator.
- o Requires ISS certification.

Fluid Processing Apparatus (BioServe)

- o Non-adherent, previously used for primary and tissue cultured cells.
- o Variable volume.
- o No medium change out, pre-filled preflight.
- o With and without gas exchange membrane.
- o Manual or automated injection of secondary solutions.

- o Flown on ISS provides temperature and humidity data.
- o Can be used with the Group Activation Pack (BioServe).
- o Uses CGBA (BioServe).

BioServe Culture Apparatus (BCA; BioServe)

- o Gas permeable bags (4 bags total; 32 ml per bag).
- o Syringe system for injections.
- o Variable volume.
- o Four (5 ml) vacutainers for sample collection.
- o Can charge sealed GAP with required gas mixture or use ambient Module air (GE-GAP).
- o No gas exchange; a version does exist that allows gas exchange, but BioServe does not advertise its use anymore.
- o Fully automated system.
- o ISS certified.
- o Uses CGBA (BioServe).

GAP-Derived Cell Culture (BioServe)

- o OptiGAP used to house OptiCells™; can use the GAP hand pump system for injections.
- o GE-GAP (gas exchange); gas exchange member in GAP housing.
- o ISS certified.
- o Uses CGBA (BioServe).

Acronyms

3-D	Three-dimensional
ABRS	Advanced Biological Research System
BCA	BioServe Culture Apparatus
BSTC	Biotechnology Specimen Temperature Controller
CASIS	Center for Science in Space
CBEF	Cell Biology Experiment Facility
CCM	Cell Culture Module
CEU	Cell Experiment Unit
CGBA	Commercial Generic Bioprocessing Apparatus
CHab	Culture Habitat
EET	Experiment elapsed time
ESA	European Space Agency
EXPRESS	Expedite the Processing of Experiments to the Space Station
FSB	Fundamental Space Biology
GAP	Group Activation Pack
GE-GAPS	Gas Exchange - Group Activation Packs
GSM	Gas Supply Module
HARV	High-aspect rotating vessel
ISPR	International Standard Payload Rack
ISS	International Space Station
ISSNL	ISS National Lab
LEO	Low-Earth orbit
LET	Linear energy transfer
MOBIAS	Multiple Orbital Bioreactor with Instrumentation and Automated Sampling
MSG	Microgravity Scientific Glovebox
NASA	National Aeronautics and Space Administration
NRC	National Research Council
NSPIRES	Solicitation and Proposal Integrated Review and Evaluation System
PBLs	Peripheral blood lymphocyte cells
PI	Principal investigator
PSA	Prostate-specific antigen
RNA	Ribonucleic acid
RWV	Rotating wall vessel
STLV	Slow-turning lateral vessel
STS	Space Transportation System (space shuttle)

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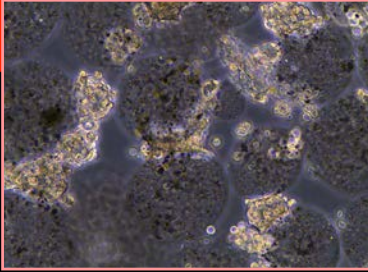
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