

# THE BIOTECHNOLOGY FACILITY FOR INTERNATIONAL SPACE STATION

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## ABSTRACT

The primary mission of the Cellular Biotechnology Program is to advance microgravity as a tool in basic and applied cell biology. The microgravity environment can be used to study fundamental principles of cell biology and to achieve specific applications such as tissue engineering. The Biotechnology Facility (BTF) will provide a state-of-the-art facility to perform cellular biotechnology research onboard the International Space Station (ISS). The BTF will support continuous operation, which will allow performance of long-duration experiments and will significantly increase the on-orbit science throughput.

With the BTF, dedicated ground support, and a community of investigators, the goals of the Cellular Biotechnology Program at Johnson Space Center are to:

- Support approximately 400 typical investigator experiments during the nominal design life of BTF (10 years)
- Support a steady increase in investigations per year, starting with stationary bioreactor experiments and adding rotating bioreactor experiments at a later date
- Support at least 80% of all new cellular biotechnology investigations selected through the NASA Research Announcement (NRA) process

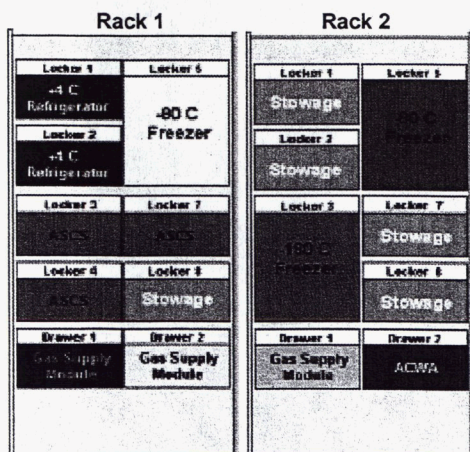


Fig. 1. The proposed layout of the Biotechnology Facility dual-rack component space.

## 1. CAPABILITIES OF THE BTF

The BTF will employ several methods to increase scientific return over the Cellular Biotechnology Operations Support System (CBOSS), which was an interim platform for cellular biotechnology research on ISS.

- Automated experiment operations - to reduce crew time requirements and to standardize experiment procedures

- Modular components - to allow sequential and continuous experiment operations without cross-contamination
- Increased cold storage capability (+4°C, -80°C, -180°C)
  - Storage of frozen cell culture inoculum - to allow sequential investigations
  - Storage of post-experiment samples - for return of high quality samples
- Increased number of cell cultures per investigation, with replicates - to provide sufficient number of samples for data analysis and publication of results in peer-reviewed scientific journals

### 1.1 Core Functions

The BTF will be a complete research laboratory facility that is equipped with the resources necessary to conduct cellular biotechnology experiments in the microgravity environment on the International Space Station. The BTF components provide four core functions:

#### 1.1a Cell Culture Incubation

The BTF will provide a controlled environment for the cultivation of cells into three-dimensional tissues. These cell and tissue cultures, as well as the conditioned media, will be the primary source of sample material available to investigators for analysis.

#### 1.1b Experiment Support

The BTF will provide the materials and hardware required to support cell and tissue culture experiments, including:

- Production of cell culture media
- Supply of tissue culture grade gas
- Cold storage
  - +4°C: storage of culture media and other supplies, post-experiment samples
  - -80°C: storage of media supplements, post-experiment samples
  - -180°C: storage of cell culture inoculum
- Ambient storage for experiment supplies

#### 1.1c Experiment Assessment

The BTF will provide capabilities to assess cell and tissue culture experiments. Capabilities include sensors in BTF components and use of a portable clinical blood gas analyzer.

#### 1.1d Command and Control/Data Handling

The BTF will provide the command and control functions required to monitor and control the operation of BTF racks, to monitor the health and status of the BTF sub-rack components, and to partition resources to ensure that the maximum science return is achieved. The BTF will also provide experiment command and control/data

handling functions. These will include telemetry downlink and real-time command uplink from BTF Operations Support Facilities.

## **2. COMPONENTS OF THE BTF**

### **2.1 Biotechnology Facility Components**

The BTF will consist of two Expedite the Processing of Experiments to the Space Station (EXPRESS) racks containing the sub-rack elements required to conduct cellular biotechnology research. Experiment hardware will feature a "high through-put" cell and tissue culture system and several pieces of experiment-support hardware. The Automated Stationary Culture System (ASCS) is designed to accommodate multiple cultures and multiple investigations simultaneously. Additionally, the ASCS will allow end-to-end increment operation on ISS. Experiment support hardware will include: a water purification and media mixing system to produce cell culture media on-orbit (ACWA), a gas supply module (GSM), and dedicated cold storage facilities.

#### **2.1a Automated Stationary Culture System (ASCS)**

The ASCS consists of two major components: the Thermal Carrier and Cell Culture Modules. The Thermal Carrier provides +4°C storage for experiment supplies and preserved cultures. Cell Culture Modules (CCM) contain an insulated micro-incubator to provide appropriate environment for incubation of cell and tissue cultures. The ASCS contains four Cell Culture Modules per unit, and each CCM contains 3 cell culture vessels. Cell culture vessel volume is 10 ml per bag and samples may be either fixed cell cultures and/or media samples. The incubation temperature set point range is +25°C - 45°C ± 1°C. CO<sub>2</sub> set point range is 0 - 10% ± 1%. Sample fixative storage temperature range: +4°C - 30°C ± 1°C. The ASCS has sensor capabilities for pH, CO<sub>2</sub>, and incubation and cold storage temperatures. The ASCS has the following automated capabilities: media exchanges, low-shear mixing of culture vessel contents, pH monitoring, media sampling, culture wash and (semi-automated) fixation. Manual capabilities include a sample port on each cell culture vessel for sampling on an as-needed basis.

#### **2.1b Automated Culture Water Assembly (ACWA)**

The ACWA is designed to process water from any ISS potable water source into cell culture grade water. This is accomplished through an automated capability to obtain ultra-pure water, then mix the water with liquid media concentrate and pre-thawed media supplements to produce cell culture media. Additionally, the ACWA dispenses the produced media into the container(s) required by the BTF cell culture hardware. Wetted components are replaced to prevent cross-contamination from one media production to the next. The ACWA permits on-orbit filter change-out for extended duration operation.

#### **2.1c Gas Supply Module (GSM)**

The GSM provides tissue-culture grade gas for BTF cell culture hardware and experiments. The gas is pre-mixed on the ground, and will be between 0 and 10% CO<sub>2</sub>, depending on the investigator's requirements. The GSM contains two gas output ports capable of simultaneous operation and has a flow rate delivery of a maximum of 1000 standard cubic centimeters per minute (SCCM). Additionally, it provides quick disconnect regulated gas pressure of 40 ± 7 psig and front panel displays for the storage pressure and the delivery pressure.

#### **2.1d Cold Stowage**

Currently, the BTF is planned to provide dedicated cold storage facilities for refrigeration and cryogenic preservation of inoculum, media, and samples. These cold storage facilities are being developed by the ISS Payload Office. Capabilities include +4°C, -80°C, and -180°C with both internal and external temperature sensing capabilities.

#### **2.1e BTF Operations Support Facilities**

BTF real-time operations support will be coordinated from two dedicated workstations in the Telescience Support Center (TSC) at Johnson Space Center's Mission Control Center (MCC) and will provide ground to ground voice loops for communicating with the Payload Operations Integration Center (POIC) at Marshall Space Flight Center, ISS space to ground voice loops for monitoring crew communications, ISS payload downlink telemetry, ISS video downlink, and ground commanding. Additionally, an alternate site for operations and experiment support will be established at Wyle Laboratories in Houston, TX and investigators will be able to monitor appropriate downlink data from a remote site through a password protected website.

## **3. DEVELOPMENT STRATEGY**

### **3.1 Concepts Proven In Development Units**

In the Preliminary Design phase, tests on the BTF have been performed at the system, subsystem, or component level to show that the specific design approaches are acceptable to meet the functional and/or performance requirements of the hardware, firmware, and software. Development units (proof of concept models and test beds) composed of hardware and software that perform the basic functions of an engineering unit, have been constructed and results summarized below.

#### **3.1a Concepts Validated in ASCS Model and Breadboards**

The ASCS proof-of-concept was successfully demonstrated in an incubation chamber housed in a +4°C volume that maintained the appropriate environment to support viable cell cultures (37°C, 5% CO<sub>2</sub>). Cell lines tested included both robust, fast growing cell lines, sensitive cell lines, and cell lines that utilize microcarrier beads. Using this proof-of-concept model, the automation of stationary cell culture operations was also validated; demonstrating successfully the rapid thaw of frozen inoculum, automated media exchanges, mixing of culture vessel contents, collection of media samples, cell

culture wash and fixation, and short-term preservation of fixed cultures at +4°C.

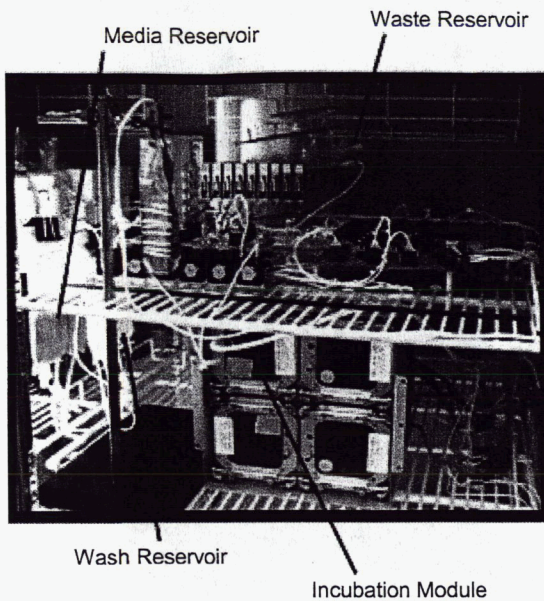


Fig. 2. ASCS Proof of Concept Model

### 3.1b ACWA Test Beds

The ACWA proof-of-concept was successfully demonstrated in a water purification test bed, a media mixing test bed, and a complete ACWA test bed. In the water purification test bed, an ion exchange resin and activated carbon was used to remove biocides present in ISS water sources, and biological filters were used to remove endotoxins and bacteria. Test results showed biocompatibility of purified water, and was later confirmed by gene array analysis on cells cultured in media produced with ACWA-purified water. The media mixing test bed used lab supplied water mixed with concentrated media and supplements. Cells cultured in ACWA-mixed media were evaluated for growth, morphology and nutrient utilization and were similar to ground controls. Additionally, the media mixing test bed demonstrated the bubble removal capability. The complete ACWA test bed was used to purify water, mix with concentrated media and supplements, and showed that cells cultured in ACWA-prepared media were similar to controls for growth, morphology and nutrient utilization.

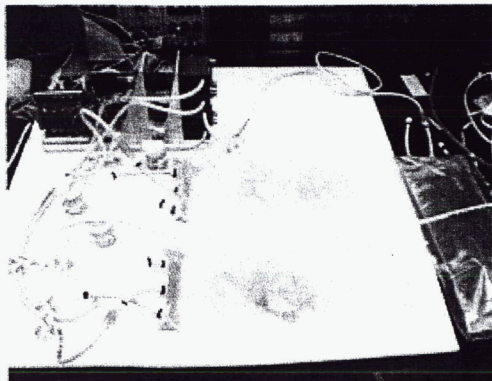


Fig. 3. ACWA Media Mixing Test Bed

## 4. CELL GROWTH IN EARLY ENGINEERING UNITS

### 4.1 Cells Cultured in Media Prepared with ACWA

Initial tests in the ACWA demonstrated that both MIP-101 and human lung fibroblasts (WI-38) showed similar cellular growth, morphology, and glucose utilization between cells cultured in ACWA media and in control media. The ACWA mixing test bed was utilized to prepare GTSF-2 media by mixing cell culture grade water with 3X liquid media concentrate and media supplements. Human colorectal carcinoma cells (MIP-101) were cultured in the ACWA prepared media and in lab-prepared control media for six days.

### 4.2 Cells Cultured in ASCS Model

Initial tests in the ASCS Proof of Concept Model demonstrated that an insulated incubation chamber housed at +4°C will support viable cell cultures.

MIP-101 human colorectal carcinoma cells were successfully cultured in the ASCS for seven days. Cellular growth, morphology and nutrient utilization were similar to control cultures housed in a standard laboratory incubator. Within 24 hours of inoculation, MIP-101 cells formed three-dimensional aggregates. As the cells proliferated, the aggregates increased in size and number. Following the test, aggregates were exposed to MTT reagent to confirm cell viability. Metabolically active cells appeared purple. In additional tests, EMS-3 Rauscher murine erythroleukemia cells were successfully cultured in the ASCS model.

### 4.3 Cells on Microcarrier Beads in ASCS Model

Subsequent tests in the ASCS Proof of Concept Model demonstrated that the key concepts required to perform a complete cell culture experiment in the ASCS can be successfully achieved.

- Rapid thaw of frozen inoculum
- Dilution and removal of cryopreservation agent
- Culture incubation (37°C, 5% CO<sub>2</sub>)
- Automated media exchanges
- Automated collection of media samples
- Automated wash and fixation of cell cultures
- Short-term storage of fixed cultures at +4°C

MIP-101 human colorectal carcinoma cells were cultured with microcarrier beads in the ASCS for seven days. Cellular growth, morphology and nutrient utilization were similar to control cultures housed in a standard laboratory incubator. Cells were washed with PBS and fixed with formalin. Following the test, fixed cells were stained with hematoxylin to confirm preservation of cell morphology.

## 5. ENGINEERING DATA

### 5.1 Computational Fluid Dynamics Analysis

A mixing procedure is necessary for the stationary culture vessels to distribute inoculum, fresh media, or other treatments uniformly throughout the cell culture vessel volume. Mixing will also provide an even distribution of nutrients throughout the cell culture vessel, which is essential to promote cell growth and aggregate formation. A kneading-like mixing protocol that transfers the cell culture media from one side of the culture vessel to the other has been proposed for use in the Automated Stationary Culture System (ASCS). This protocol has been modeled using FLUENT™, a computational fluid dynamics tool. The mixing process will produce low mechanical stress levels and a laminar flow to avoid damage to the shear sensitive cells. The mixing protocol will use a low frequency of oscillation (0.1 Hz) and a 1.5 ml volume displacement to produce a 0.0024 dynes/cm<sup>2</sup> maximum shear stress, which is 60-fold less than the critical 0.15 dynes/cm<sup>2</sup> level which harms cells. Preliminary CFD analysis has demonstrated that this protocol will result in effective mixing within minutes.

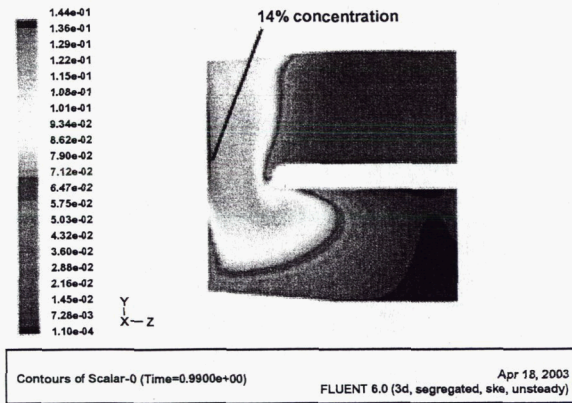


Fig. 4. After 3 Cycles, Time= 10 seconds

These images show the results of a fluid dynamics analysis conducted using FLUENT™ software to simulate the proposed method of mixing within the culture vessels. The simulation tracks mixing of relative oxygen concentration from 100% to 14% over three cycles. This preliminary data is based on saturated oxygen fluid modeling. Additional analysis is required to evaluate cells, cell aggregates, and media agent mixing.

## 6. FIELDS OF INVESTIGATION

### 6.1 Major Investigation Areas

It is anticipated that the BTF will be utilized by ISS investigators in the following areas of study:

3-D Tumor Modeling	Membrane Dynamics
Angiogenesis	Microencapsulation
Apoptosis	Models for Disease
Biomaterials	Molecular Biology
Biosensors	Pharmaceutical Studies
Biosentinels	Proteomics
Cellular Countermeasures	Radiation Modeling
Cellular Locomotion	Signal Transduction

Cellular Metabolism  
Cellular Physiology  
Differentiation  
Genomics

Tissue Engineering  
Tissue Morphogenesis  
Tissue Transplantation  
Toxicology

Some typical goals for expected microgravity investigations would be:

- Compare activation and/or deactivation of genes
- Examine alterations in cell motility
- Examine changes in cellular structure
- Examine changes to signal transduction pathways
- Examine alterations to enzyme-mediated chemical activity
- Observe apoptotic changes
- Construct complex functional tissues
- Compare types and quantity of protein production

### 6.2 Application of Findings

Microgravity can be used as a powerful research tool. Cellular responses to the stress of exposure to microgravity can provide insight into basic cellular mechanisms. The microgravity environment can also be used to facilitate construction of functional tissues that either can not be assembled in 1G or that are difficult to grow on Earth. Knowledge gained from cellular biotechnology microgravity experiments can be applied to disease fighting efforts on Earth and aid in the genesis of potential cellular countermeasures for human space flight.

## 7. CONTACT INFORMATION:

For additional information on NASA's Biotechnology Facility for ISS, please contact the BTF Project Manager Katy Hurlbert, Ph.D, [katy.hurlbert@jsc.nasa.gov](mailto:katy.hurlbert@jsc.nasa.gov) or the BTF Project Scientist, Thomas Goodwin, Ph.D. , [thomas.goodwin@jsc.nasa.gov](mailto:thomas.goodwin@jsc.nasa.gov). Also, you can find information at the Biological Systems Office Website at: <http://sbsd.jsc.nasa.gov/bsa>