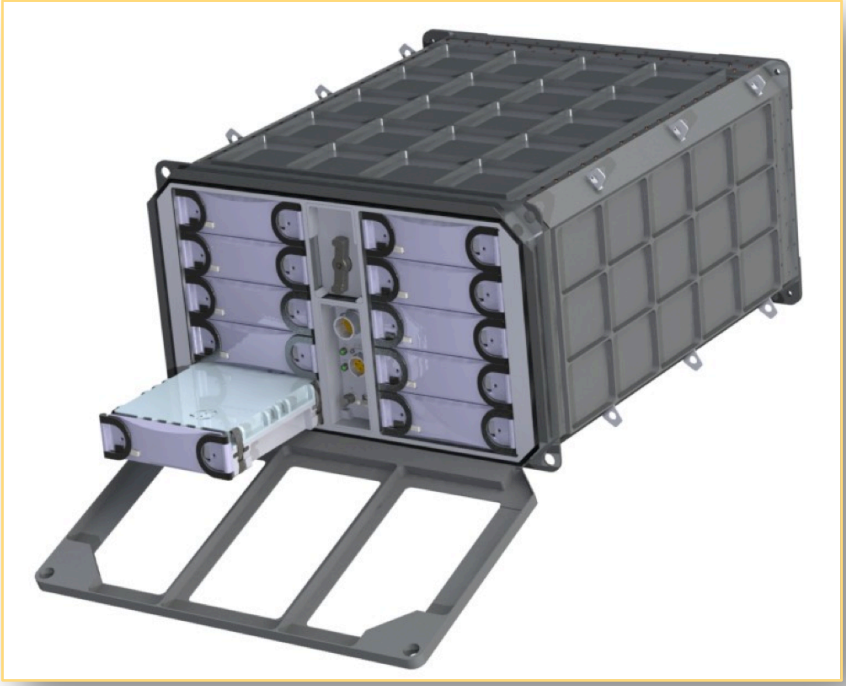
National Aeronautics and
Space Administration

NASA Bioculture

Overview

The Bioculture System is an automated and Crew-accessible cell culture and experiments system that provides controlled maintenance and growth of biology in the microgravity environment of space. This state of the art system incorporates independent perfu-sion based cell culture bioreactors and computer controlled fluidics to allow for multiple automated experiment manipulations. The automation includes standard laboratory culture maintenance such as temperature control, O_2/CO_2 delivery, and media change out. Control is further enhanced to include automated injections and/or fraction collections programmed as part of the experiment plan. The Bioculture System provides 10 indepen-dently controlled experiment cartridges each with independently controlled temperature set points and independently controlled refrigerator set points. The integral refrigerator compartments are used to extend the life of heat liable nutrients, additives and fixatives as well as improved maintenance of sample fraction collections. This system incorpo-rates flight-proven designs and lessons learned during the extensive Cell Culture Module (CCM) flight series. Most importantly, the Bioculture System addresses a critical need for ISS National Lab academic and commercial space bioscience research - a containment system that allows for initiation, intervention, and analysis of experiments on-orbit.



Cell Culture Module Heritage

The Bioculture System is specifically designed to emulate the Cellmax perfusion culture system. The primary component is the hollow fiber bioreactor, now commercially available from Fibercell Systems, Inc. http://www.fibercellsystems.com. Hollow fiber bioreactors are designed to deliver nutrients and remove waste via multiple, tightly-packed perfusion fibers. These selectable, permeable-weight fibers efficiently deliver nutrients and remove waste from the cells growing in the extracapillary space while preventing perfusion shear forces on the cells. The hollow fiber system is particularly suited for micro-gravity cell culture where nutrient transfer is limited to diffusion. The increased surface area and low shear perfusion ensures that cells are receiving sufficient nutrients and gas to grow unabated.

A principal advantage of the Bioculture System is the automation of standard laboratory processes such as media feeds, removal of waste, sample collection, protocol additions, and other additions such as antibiotics or growth factors to the culture. Each of the 10 cartridges is equipped with 16 fluid diverters plumbed into the flow path. These diverters can be automatically sequenced to execute a variety of experiments plans. Additionally, the Bioculture System pump is bi-directional, adding additional flexibility to mixing and sampling from within the flow path.

Beyond the automated features, the Bioculture System is designed for glovebox assisted astronaut breach and re-establishment of the levels of containment. This allows for on-orbit culture initiation from frozen stores and recovery of cells/samples for storage and transport in low temperature freezer systems. Glovebox assisted procedures enables real time analysis via the ISS National Laboratory analytic assets. A principal advantage of the Bioculture System is that it is a platform system that can be re-configured and customized to meet specific scientific objectives.

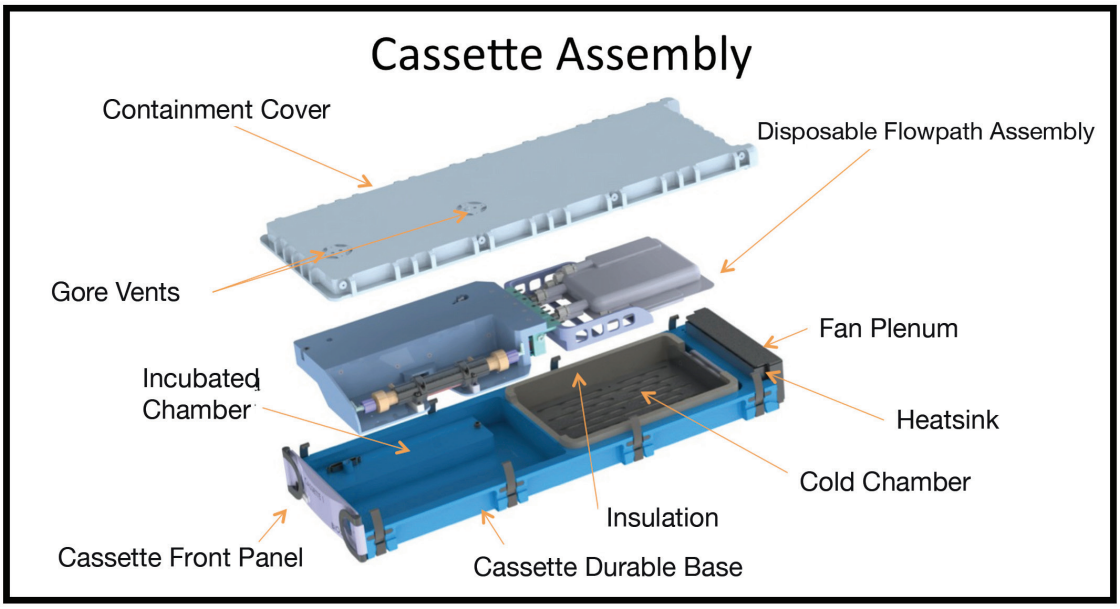
Generally, cells that can be grown in flasks can be adapted to the hollow fiber system. Below are examples of previously flown cells:

- **Skeletal myoblasts**
- **HFOB cells (human osteoblast-like cells)**
- **Chicken osteoblasts**
- **Human osteoblasts**
- **Primary hamster fibroblast**
- **Human colon carcinoma cells**
- **Human lung fibroblasts**
- **Chick embryo osteoblasts**
- **Bovine Aortic Endothelial Cells**
- **Adipose-derived Mesenchymal Stem Cells**
- **Stem cells (hematopoietic; adipose-derived)**
- **Stem cells (mouse embryonic)**
- **Rat skeletal muscle cells (L8)**
- **Rat osteoblasts**
- **Mesenchymal cells (cartilage)**
- **Bone cells of the osteoblast family (ROS 17/2.8 cells)**
- **Elastin Heterografts**
- **Rat glial tumor cells (C6)**
- ***Salmonella typhimurium***

The Bioculture System consists of a main docking rack housing the power/communication module, gas module, and 10 experiment cassettes. Within each cassette, the experiment is configured as a self-contained, independent, disposable insert. The disposable insert is a one-piece, sterile barrier flowpath with integral bioreactor, pump, media reservoirs, sump, oxygenator, diverter and manifold valving and preheat reservoir. The disposable insert defines the organization of all pumps, valves, flowpath connec-tions and bioreactors required for an experiment.

Cassette

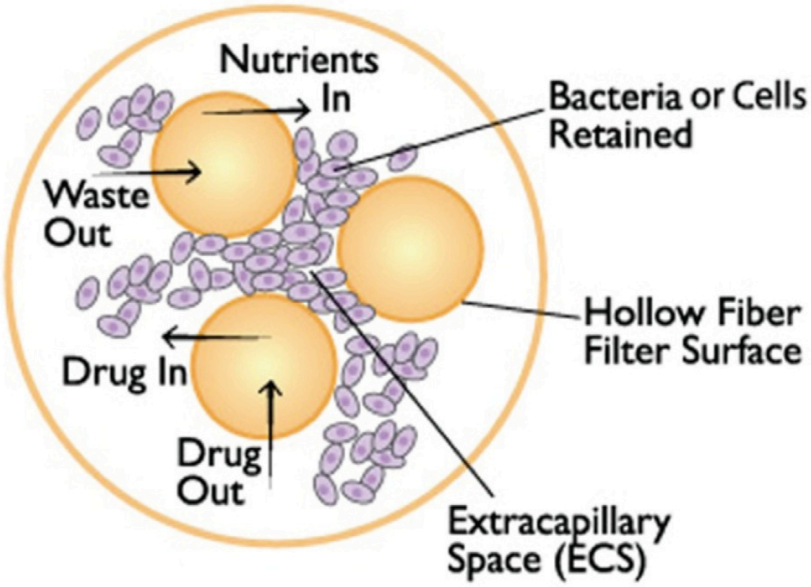
The configuration of each flow path can be customized based on the requirements of the experimental plan. The closed flow path acts as a first level of containment for biologics and hazardous materials that may be found within. The cassette disposable is secured into the cassette durable which provides power, communication, one-way gas mating, incubator and refrigerator compartments, and structural support for the second and third containments. This system will allow the crew to remove a cassette from the cassette rack and transport it, with the contained disposable fluidics/bioreactor, to the Micrograv-ity Science Glovebox (MSG). Within the MSG, the crew will be able to remove the second and third containment levels to access and manipulate the cell experiment.



Possible manipulations include, but are not limited to, removing components of the flow path (e.g., sample bags), performing injections into the flow path, and moving various detachable items or the en-tire cassette disposable from the MSG to scientific equipment available at the ISS National Lab.

The Bioculture System will include standard laboratory fixing solutions, maintained in the refrigerator compartment of the cassette. The fixatives are automatically delivered and mixed via the flow/reverse and trans-membrane flow capabilities of the system. For cell culture initiation from frozen stores, TGI has designed a simple, integrated cryo-preservative removal and bioreactor inoculation system for ef-ficient/sterile bioreactor seeding while in orbit.

Fibercell Bioreactor



The unique properties of the hollow fiber bioreactor make it an ideal choice for experiments conducted in microgravity on the International Space Station. The smaller cartridges make a multi-chambered, space efficient design of the Bioculture System possible while providing the best environment for successful cell culture. To better understand the advantages of the design, some detail of the schematics is required. The bioreactor contains small hollow fibers shaped like drinking straws that are each approximately 200 microns in diameter. The fibers are sealed inside a cartridge so that the medium pumped though the end of the cartridge will flow though the inside of the fibers while the cells are being grown on the outside of the fibers. This framework allows the fibers to create a defined semi-permeable barrier between the growing cells and the flowing medium. It also allows the cells to constantly exchange waste for nutrients, unlike a typical lab setting where the waste and nutrients must be monitored and exchanged manually. This design allows the nu-trients to reach the cells as fast as viability and reducing apoptosis. The cells are attached to a porous support, splitting of the cells is not required. Bioreactors have an extra-capillary space volume of 2.5 milliliters containing a surface area for culturing of 75 square centimeters and a maximum cell capacity of 10^8 cells. A variety of larger hollow fiber bioreactors can be used as well depending on the nature of the experiment.

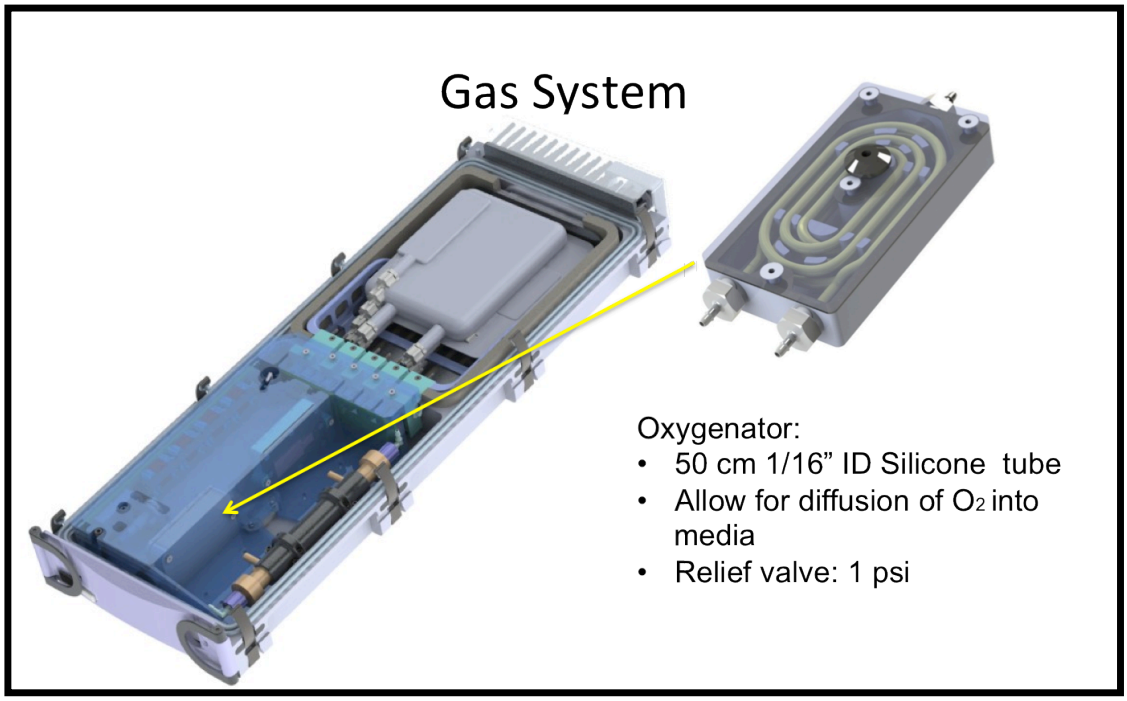
Incubation/Refrigeration

In most cases, the Bioculture System will be programmed to maintain a constant 37°C environment. However, it is possible to program temperature set point of the incubator side of the cassette from ambient temperature to 45 degrees C or higher. Also, the temperature set point can be modified near real time via ground commanding. Each cassette includes a 200ml refrigerator ≅5°C compartment meant to store heat liable media, additives (growth factors, antibiotics, etc.) and sample/fraction collections. The temperature set point of the cold compartment can be programmed from 5°C to ambient. Also, the temperature set point can be modified near real time via ground commanding

The 200 ml cooling chamber may be used to house the experiment biology where reduced temperature quiescence is desired for such models as embryos, bacteria or other reduced metabolic protocols.

Gas Exchange

The Bioculture System carries its own medical grade gas supply of 20% O_2, 5% CO_2, and balanced N_2. The gas supply is metered and distributed via a manifold to the independent experiment zone oxygenator. The oxygenator employs a series of silicone flow loops to provide gas exchange across the semipermeable flow path tubing. The oxygenator is supplied with new gas at a cassette specified rate via the gas distribution manifold. The sealed, one-way gas containment prevents undesirable gases from entering the oxygenator.



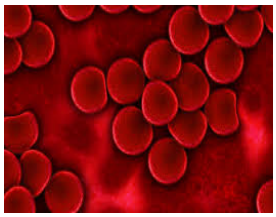
Media Delivery

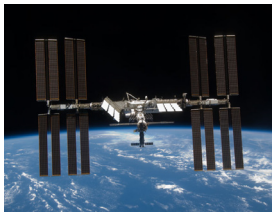
The media delivery scheme, which features a closed loop flowpath design, provides the ability to achieve most cell maintenance operations and science manipulations. The flow of liquid supplies cultures with nutrients and dissolved oxygen required for stasis and allows for the introduction of other fluid reagents, including stimulants, hormones and fixatives. This fluid flowpath is generally available in two different nutrient delivery options.

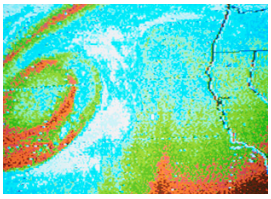
The first option is a recirculating flowpath, in which media flows through the media bag, oxygenator, pump, and cartridge before returning to the bag. In this configuration growth factors and other feed-back-related products will accumulate in the recirculating bag.

The second option is an intermittent feed, which periodically pumps fresh medium into a short flowpath that recirculates in the same manner as the recirculating flowpath. This media is eventually diverted to a sump and is replaced with fresh medium. This method also offers periodic replacement of the entire media volume.

An advantage of either scenario is that media containing heat-labile agents (e.g. ascorbic acid, antibiot-ics, and serum) can be maintained in the refrigerated compartment, pre-heated and injected into the delivered flowpath system before use.

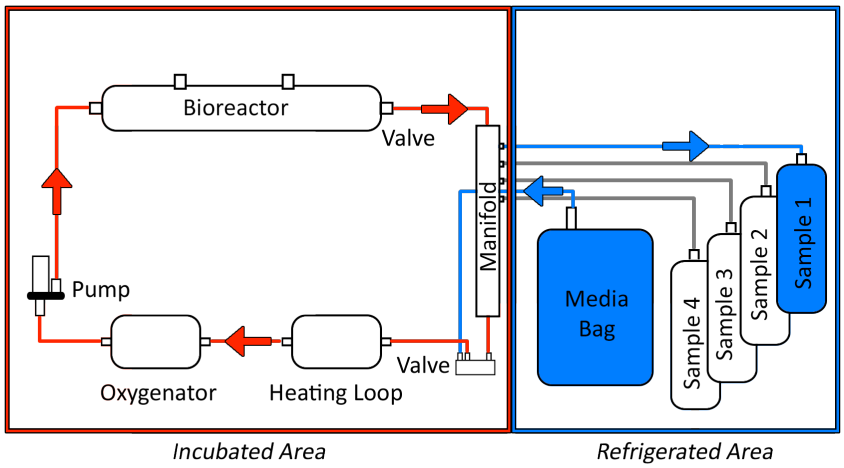






Injections and Sampling

The injection system can be used to expose the samples to stimulants, drugs, hormones, chemical labels, and/or fixatives. Injections can be paired with media collections to test various parameters including radiolabel incorporation into cellular products, and the effects of drugs and stimulants over the course of the experiment. Sequential injections of stimulants and fixatives can give an investigator an indication of cellular activity over time.



Media fraction collection allows for the testing of the presence or absence of a particular media component, metabolite, or cell product at a precise time point, and is often paired with the injection system. Periodic measurement of these substances allows a determination of metabolic activity to be assessed over time during any stage of the experiment. A technique currently in development is sampling from the cell containment space. This will allow the harvest of non-adherent cells, proteins, and other products that might be too large to pass through the hollow fiber pores of the cartridge.

Real Time Monitoring and Ground Control Access

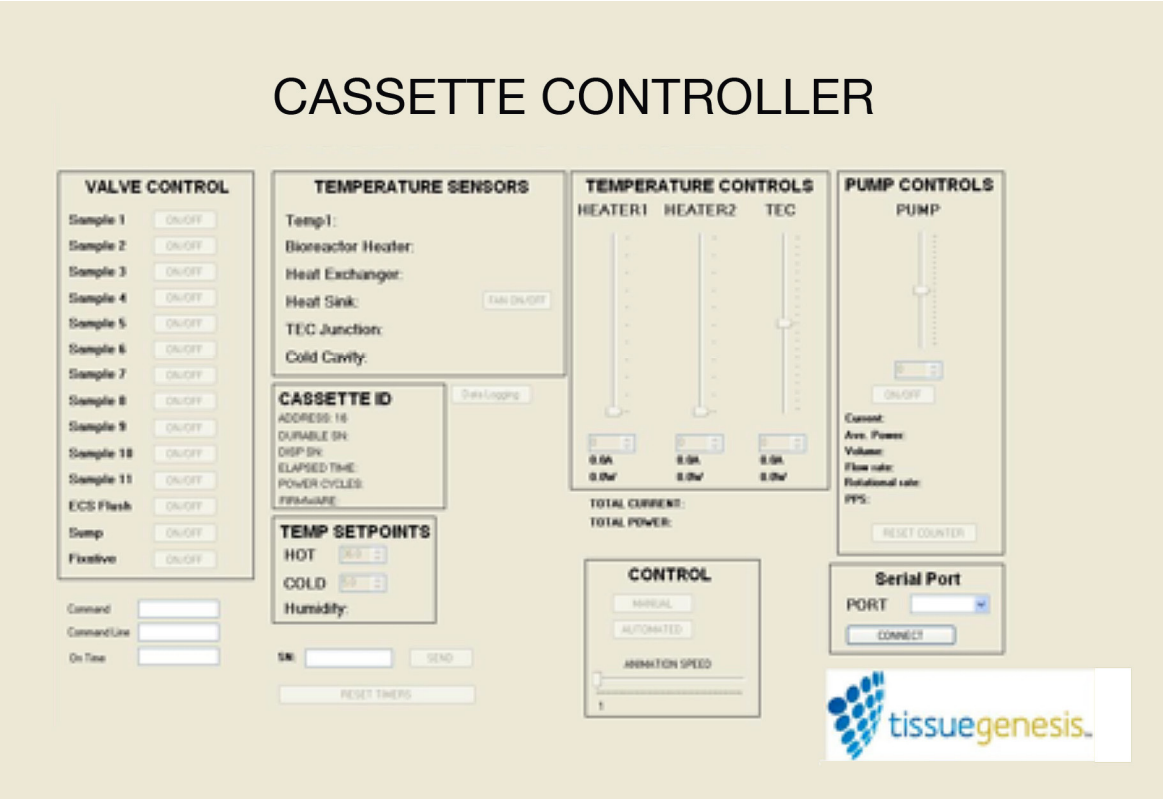
Using the custom controls, the experiment is monitored and commanded from a ground site. This allows the investigator real-time status updates and enables experiment protocol set-point and automated event schedule near real time modification by ground commanding as needed or may be dictated by launch, initiation, or astronaut timeline constraints.

Valve Control

Temperature Controls

Pump Controls

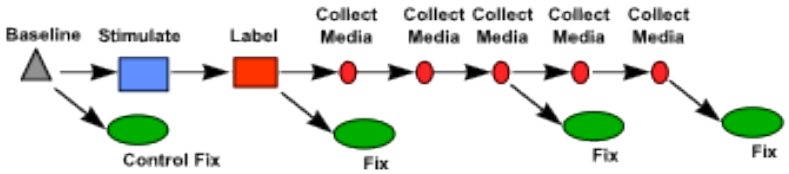
Temperature Setpoints



Experiment Design and Development

Typical Experiment Design Example

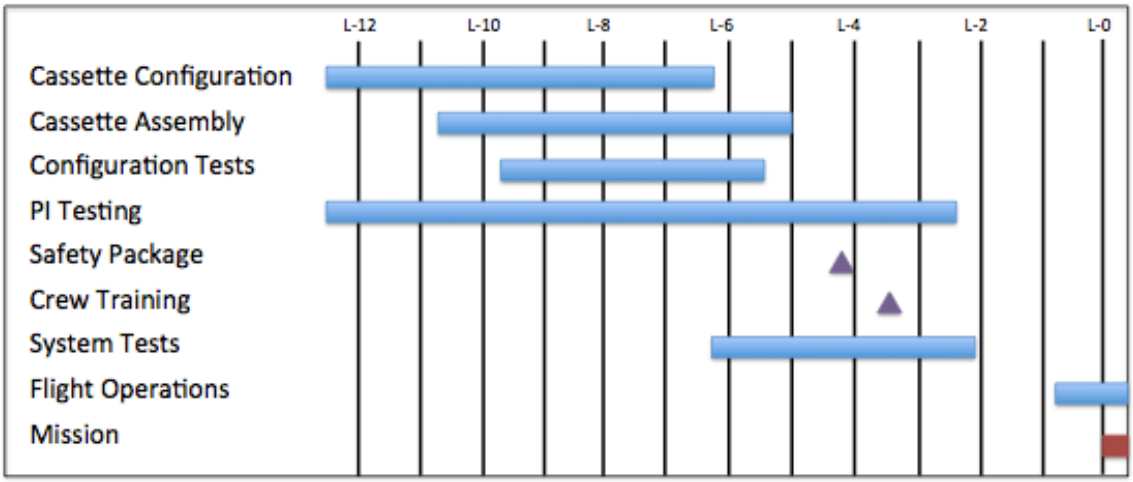
A typical Bioculture System experiment will utilize a set or series of pre-programmed events to accomplish experiment objectives. An experiment can easily combine periodic on-orbit media sampling and fixation of individual specimens (shown below). A typical study might examine the effects of in-flight hormone stimulation by collecting media fractions at pre-set intervals post-exposure. For example, cells may be automatically sampled at specific time points or process by fixation. Injection of the hormonal treatment would be automated. Crew operations may include initiation of culture on-orbit and specimen transfer to ISS MELFI for long duration stowage.



Mission

Top Level Experiment Timeline

Investigators should expect to spend one year conducting ground studies and science/hardware compatibility testing prior to flight. However, the actual duration (longer or shorter) for ground studies and tests will depend on the complexity of the experiment, cell type, and if the specimens are previously-flown or grown in a hollow fiber system. Once selected for flight, compatibility with the Bioculture System is assessed and hard-ware and science requirements are defined. Six months to a year may then be spent adapting the experi-ment to the bioreactor system and optimizing growth conditions. Hardware configuration and compatibility testing in the investigator's laboratory follows. When the science and equipment are performing appropriate-ly, a full scale and mission duration test may be performed using the fully flight configured Bioculture System at an appropriate test facility (e.g. NASA ARC or NASA KSC). The mission duration test aids both scientists and engineers in mission and contingency preparation, including final biocompatibility tests, ground baseline experiment data collection, sterile processing concerns, procedures, pre-flight timelines, mission delays/scrubs, and the logistical concerns of "taking an experiment on the road."



Example timeline for experiment and payload definition development

Customizable Options

Investigators have a wide variety of experiment options available to them. Each of these capabilities is automated and maintains the integrity of the sterile flowpath.

- **Variety of bioreactor cartridge systems**
- **On-orbit initiation of culture from frozen or cold stow vials (automated or Crew tended)**
- **Launch and return of active cultures in the Cassette**
- **Crew activities for specimen processing, analysis, and cold/frozen stowage transfers**
- **Return of samples in the cold chamber of the Cassette**
- **Constant or variable environmental temperature setting**
- **Media delivery (recirculation, intermittent delivery, or media source change)**
- **Programmed additions of drugs, hormones, stimulants, radioactive labels, etc.**
- **On-orbit fixation at various time points**
- **Automated collection of conditioned media samples at a variety of time points**
- **Crew access to incubation and cold compartments for manual operations**
- **Approximately 200 mls. of ≅5°C of cold storage space**

Investigators interested in using the Bioculture System for experimentation please contact:

Edward Austin
Bioculture System Project Manager
NASA Ames Research Center
edward.m.austin@nasa.gov
650-604-2108

Jeffrey Smith, Ph.D.
Space Biology Project Manager
NASA Ames Research Center
jeffrey.d.smith@nasa.gov
650-604-0880

Bioculture System was developed by Tissue Genesis, Inc. (Honolulu, HI) under contract to NASA

Bioculture Systems Specifications Summery

Physical

- Express rack locked soft stow
- 57 lb. control weight
- Late access payload (24 hours prior to launch)
- Triple containment of biologic and hazardous chemicals
- Power: 140 Watts max
- Concurrent flight and ground controls

Media Delivery

- Individual flowpaths (biocompatible components such as Pharmed, Silicone, Viton, Teflon, etc.)
- Delivery rates from 1 ml to 15 ml per minute
- Feeding Schedules: Continuous recirculation or preprogrammed feeding schedule
- Oxygen delivery and pH maintenance

Drug/Fixative Delivery System

- Programmed delivery schedule
- Cooled heat-labile drugs and fixatives
- Programmed injection of drugs or fixatives to the system (approved chemicals include 10% TCA, 5% paraformaldehyde, 2% glutaraldehyde, 70% ethanol, Histochoice™, ^{3}H, and ^{14}C)

Environmental Control

- Module temperature 32 - 39 + 0.2°C
- Cooling chamber temperature =5°C

O_2 and CO_2 Delivery System

- Computer controlled, high pressure solenoid distribution
- Medical grade gas mixture: 5% CO_2, 20% O_2, balance Nitrogen
- Membrane oxygenator using silicone elastomer tubing (DuPont)
- Positive pressure airflow within the system
- Programmable delivery schedule

Bioreactors

- Utilizes proven hollow fiber bioreactor technology or custom-design bioreactors
- Sustains attached and non-attached cell types, microcarriers
- Variable molecular weight cutoffs
- Captures cell metabolic products

Fluid System

The module can sustain a maximum of 10 independent flowpaths. Each fluid path may consist of the following:

- Media reservoir
- Media sump
- Microsolenoid diverter valves
- Pump (0-15 ml/min flow rate)
- Membrane oxygenator
- Space flight-qualified hollow fiber bioreactor cartridges or alternate selected by investigator and verified in system
- Biocompatible tubing that can accommodate hazardous fluids (fixatives, etc.)
- Programmed delivery schedule or continuous recirculating
- Programmed injection of drugs or fixatives to the system
- Fraction collections (10/flowpath)

System Redundancy

- Individual flowpaths
- Individual experiment cassettes
- Individual flow rates and pump control
- Individual feeding schedules
- Independent experiment program and operational control
- Independent temperature control

Electronics

- Power driver board for each cassette
- Dedicated controller and data logger for each cassette

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