Cell Science-02 (CS-02)
Payload Overview

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Approved for public release, unlimited distribution
Bioculture System

- Automated cell biology system with 10 independent cassettes
- Programmable manipulations include:
  - Adjustable media flow rate and feeding schedule
  - Adjustable gas dosing
  - Sample collection
  - Fixative injection
- Cassettes will be removed and opened in LSG to allow media changeout, sample retrieval and preservation, and initiation of new cell cultures.
Bioculture System

- 10 Independent Cassettes
- ExPRESS Rack Locker
- Power And Control Module
- Gas Supply
Power and Control Module

The Power and Control Module takes 28V Rack voltage, converts it to 12V, and uses standard interfaces for both power and data to the Rack. The Power and Control Module contains an On/Off toggle switch, a circuit breaker, Data Connector, Power Connector, and two LEDs for Power/Fault Status.
Durable Cassette with Disposable Flow Path
Disposable Flow Path

- Oxygenator
- Valves
- Pump
- Bioreactor
- Reservoir Bag
- Bioreactor Clamshell
- Media, Sump, Fixative, and Sample Bags
Cell Science Validation (CS-V)

- **Vehicle:** SpX-13
- **Dates:** December 15th, 2017 – January 13th, 2018
- **Crew:**
  - Joe Acaba performed the majority of CS-V operations
  - Maker (Scott Tingle) performed bioreactor removal
  - Nemo (Norishige Kanai) performed transfer to ISS
- **Results:**
  - 100% Science Return (8/8 Bioreactors)
  - Bioculture System validated for PI Missions (CS-02 and beyond)
  - Received positive feedback on hardware in crew debriefs
CS-V Imagery

Bag Change
Out in MSG

Gas Supply
Change Out
# CS-02 Experiment Summary

| Principal Investigators | Rasha Hammamieh, Ph.D.  
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|-------------------------|--------------------------------------------------|
| Co-Investigators        | Nabarun Chakraborty  
US Army Center for Environmental Health Research |
| Sponsor                 | National Lab Office/Center for Advancement of Science in Space (CASIS) |
| Funding Authority       | CASIS/Department of Defense (DoD) |
| Experiment Title        | Pan-Omics Approach to Characterize the Factors Involved in Mammalian Tissue Regeneration in Microgravity |
| Experiment Duration     | 21 Days in orbit – Initiated on the ground (22-23 days total) |
| Ground Control          | Synchronous Ground Control performed at KSC |
| Cell Type               | Primary mouse cells of osteoblast lineage |
| Independent Variable    | Growth factors – cells treated with one of two experimental growth factors (proprietary) or control (saline) |
New Crew Operations

Manual Feed:

- Crew uses two 60mL syringes to transfer used media to the Sump Bag, and then transfer fresh media from the Media Bag into the preheat reservoir.
- Will perform five of seven feeds manually, with two auto-feeds of smaller volume.
- Manual feeds are more crew time intensive, but ensure accurate feed volume across all flow paths.
Growth Factor Injections:

- Experimental factors (A, B, or C) are injected into Media Bags on the +4°C side of cassette.
- Factors degrade quickly at +4°C and need to be injected weekly. Need to be stowed frozen at -80°C or colder prior to use.
- Each cassette and flow path have a colored dot that matches up to a colored dot on the Growth Factor Syringe, to prevent mix-ups.
- All flow paths also receive Growth Factor D, which is a mixture of standard growth factors.
**CS-02 Timeline**

- **Launch:** SpX-15
- **Berth:** Dragon
- **Splashdown:** SpX-15

**Turnover: Powered Bioculture System (BCS)**
- Ship soft stow to JSC
- Transfer BCS from Dragon to ExPRESS
- Auto-Feed #1
- Gas Supply Change Out #1

**NLT L+5d³**
- NLT L+5d³
- L+6d³
- L+9d³
- L+12d³
- L+14d³

**L+7d³**
- L+7d³
- L+9d³
- L+13d³
- L+16d³
- L+19d³
- L+21d³
- L+22d³

**L+15d³**
- L+15d³
- L+16d³
- L+17d³
- L+19d³
- L+21d³
- L+22d³

**Manual Feed #1 and Manual Feed #2**
- Manual Feed #2, Bag Change Out #1, and Factor Injection #2
- Manual Feed #3, Bag Change Out #3, Factor Injection #4, and Manual Feed #4

**Gas Supply Change Out #2 and Auto-Feed #2**
- Gas Supply Change Out #2 and Auto-Feed #2
- Manual Feed #5

**Fixative Injection**
- Manual Feed #5
- Manual Feed #5
- Manual Feed #5

**BCS packed in foam for return**
- Fixative Injection

**Priority Return of soft-stowed BCS to ARC**
- BCS packed in foam for return

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Blue Text indicates the activity occurs in the MSG
Orange Text indicates automated activity (no crew operations)

1 = No more than 15 minutes powered off during transfers or gas supply changeouts
2 = Setup will also include items for Rodent Research-7 (RR-7) and AngieX
3 = Activity is tied to number of days since cell loading, which will nominally occur at L-1
Integration Overview

• Bioculture System operational for first 21 days after SpX-15 launch

• Bioculture System Facility Payload Integration Agreement (PIA) Letter contains the following unique agreements:
  • Use of three SSPF labs at KSC pre-launch, and continued use of Science lab after launch to support the simultaneous ground control
  • Late load of L-26 hours for powered Bioculture System
  • Bioculture System shall remain on the pad no longer than 50hrs after turnover, after which the biology and medium shall be changed out
  • 150 W powered on Dragon ascent – best effort to keep power interruptions during transfer to less than 15 minutes\(^1\)
  • Standard health and status data, and non-standard data - cabin temperature, total pressure, relative humidity, ppCO2, ppO2
  • Environmental and payload data from Dragon during ascent
  • Use of MSG for crew operations – coordinated with MSG and other SpX-15 MSG Payloads (Rodent Research-7, AngieX)
  • Priority return of unpowered Bioculture System to Ames after return to JSC.

\(^1\) = Exception 53146-0004, Bioculture On-Orbit Transfer Power Exception
Integration Overview (Continued)

- **Synchronous Ground Control at KSC**
- **Pre-flight specimen and hardware processing in the KSC SSPF**
  - Three labs are required and were coordinated through PSRD
  - Need to retain Science lab after launch to perform Synchronous Ground Control
- **On-orbit operations:**
  - Majority of crew procedures already developed and executed on-orbit for CS-V
  - Updates for new operations for CS-02 are in work with SpOC Biologicals Team cadre
  - Training Strategy: OBT
  - Real-time support at ARC: Telemetry monitoring and commanding
  - Requesting PD enablement and over-the-shoulder video during operations in MSG
- **Use of ISS facilities:**
  - ExPRESS Rack 7 Locker 1
  - Microgravity Science Glovebox (MSG)
  - Cold Stowage: MELFI, Polar, and DCB
  - Wetlab Pantry Items: Nitrile gloves
- **Post-flight science recoveries at PI laboratories**
  - Cold Stowage samples to be received at JSC by DoD Space Test Program (STP) and shipped to PI
Stowage Overview: Ascent on SpX-15

**Powered Locker**
- **Bioculture System**
  - Containing 10 Cassettes with live cells

**Cold Stowage**
- **Media Stowage Bag (x7)**
  - +4°C
  - Each Media Stowage Bag contains five Media Bags (total of 35)
  - One spare Media Stowage Bag, will be trashed if unused
- **Growth Factor Kit (x10)**
  - -80°C or colder
  - Each kit consists of 3mL syringes containing growth factors
  - Each kit contains 4 types of growth factors (A, B, C, and D)
  - Each kit contains enough growth factors for five flow paths
  - Two spare Growth Factor Kits, will be trashed if unused

**Soft Stowage**
- **Media Transfer Kit (x6)**
  - Contains 60mL Syringes, Hemostats, wipes, and other items for a Manual Feed
  - One Kit contains enough supplies for 10 cassettes
- **Bag Change Out Kit (x3)**
  - Contains empty Sump Bags, Hemostats, Wipes, and other items for Bag Change Out
  - One Kit contains supplies for 10 cassettes.
- **Bioreactor Removal Kit (x1)**
  - Contains Tube Cutter, two hard-sided Bioreactor Containers, and stowage bags
- **Sample Bag Removal Kit (x1)**
  - Tube cutter and Sample Stowage Bags
- **Gas Supply (x3)**
  - Expect to use two Gas Supplies for CS-02 and leave the third on ISS for CS-03
- **Setup Kit (x1)**
  - Items for setting up MSG (wipes, O-rings)
- **Accessories Kit (x1)**
  - Spare syringes, hemostats, caps, wipes, etc.
Stowage Overview: Descent on SpX-15

**Cold Stowage**

- **Return Bag -004 (x8)**
  - +4°C
  - Each contains five used Media Bags
- **Return Bag -005 (x6)**
  - -20°C or colder (-80°C preferred)
  - Each contains five used Sump Bags
- **Sample Stowage Bag (x2)**
  - -20°C or colder (-80°C preferred)
  - Each contains five Sample Bags
- **Return Bag -006 (x2)**
  - -80°C
  - Each contains five used Sump Bags and five Runoff Bags (HRL 2)
- **Bioreactor Container (x2)**
  - -80°C
  - Each container will have five individually bagged Bioreactors

**Soft Stowage**

- **Bioculture System**
  - Will be unpowered, removed from express rack, and packed in foam following conclusion of the experiment.
  - Will require oversize bag
  - Needs to be early retrieval at Long Beach
- **Gas Supply (x2)**
  - Used gas supplies for refurbishment
  - The third, unused gas supply will remain onboard for CS-03
Backup Slides
Hypothesis: Nearly all experiments investigating bone loss in microgravity have focused on studying bone physiological and morphological changes and identifying which cellular and molecular mechanism are affected. This tissue regeneration space flight experiment will investigate the ability of two different bone stimulating factors, Growth Factor A and B, to stimulate the growth, differentiation and related cellular functions of osteoblast cells in culture. This study hypothesizes that:

1. Growth Factor A and B will differentially impact osteoblast differentiation in 1g versus microgravity conditions and
2. Microgravity-specific biomarkers/networks associated with bone differentiation could have significant relevance in the astronaut therapeutic program.

Objectives: The objective of this study is to determine how Growth Factors A and B differentially affect osteoblasts in the different gravity conditions and to define alterations in genomics, transcriptomics, proteomics, and metabolomics patterns and networks.

- **Specific Aim 1:** Determine whether osteoblast lineage cells treated with Growth Factor A or B have altered multi-omics signatures.
- **Specific Aim 2:** Determine how gravity manipulates the multi-omics signatures of osteoblast lineage cells treated with Growth Factor A or B.