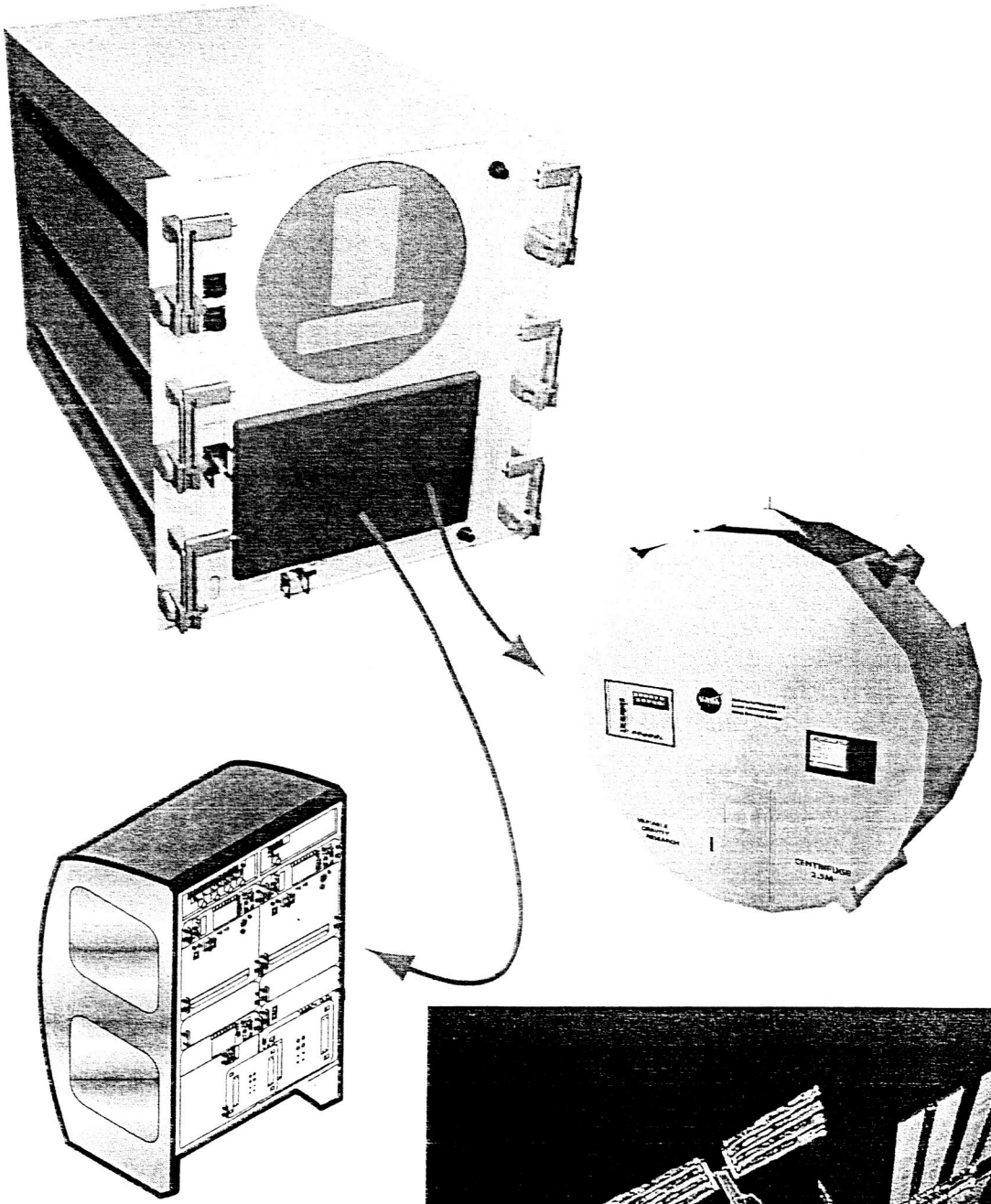
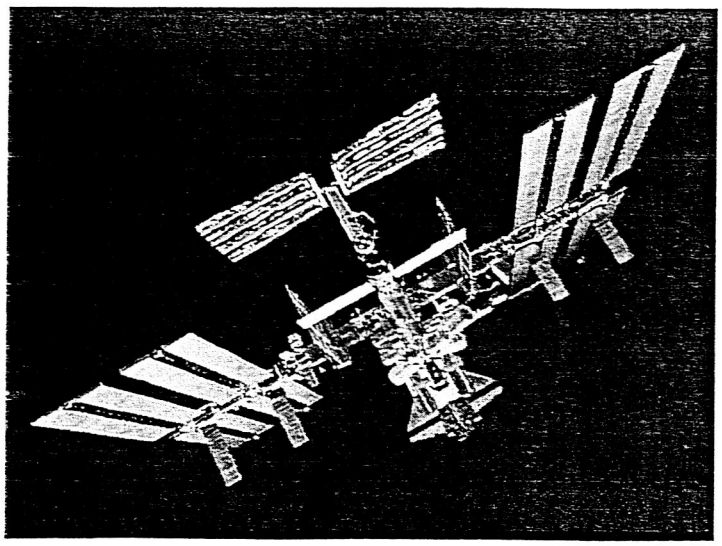
Cell Culture Unit





Cell Culture Unit Hardware Differences: Science Evaluation Unit vs Flight Unit

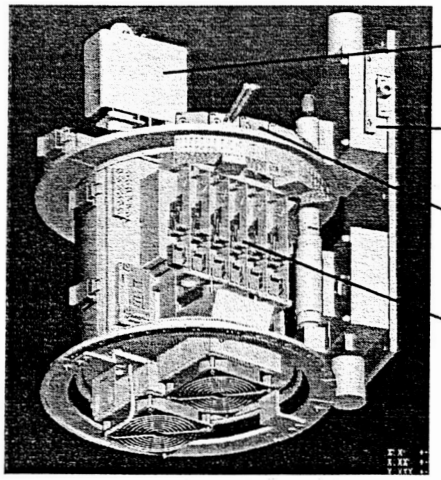
Science Evaluation Unit

Development hardware used to test cell growth in flight-like environment.

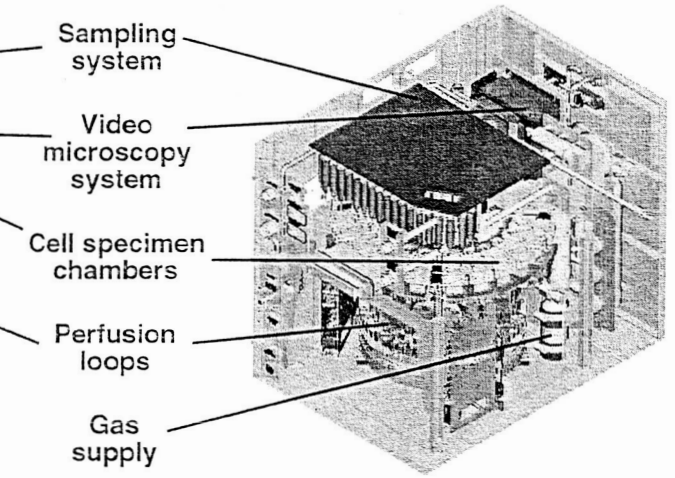
- One section (up to 6 cell specimen chambers)
- Manual sampling
- Manually operated Cell Specimen Module
- Manual Video Microscopy System operation
- Primarily off-the-shelf electronics
- Built for lab use following standard laboratory practices

Flight Unit

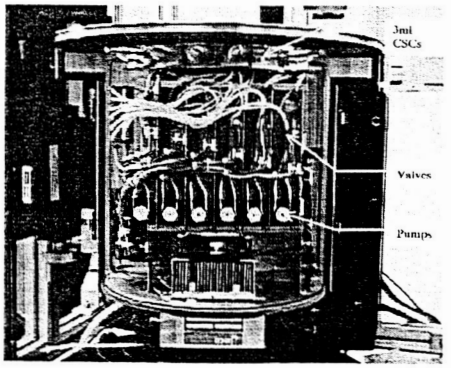
- Three sections (up to 18 cell specimen chambers)
- Automated sampling and cold (4°C) storage of samples
- Automated Cell Specimen Module
- Motorized focusing and Video Microscopy System operations
- Custom, integrated electronics
- Satisfies NASA safety requirements (containment levels, fire suppression, wiring, etc.)



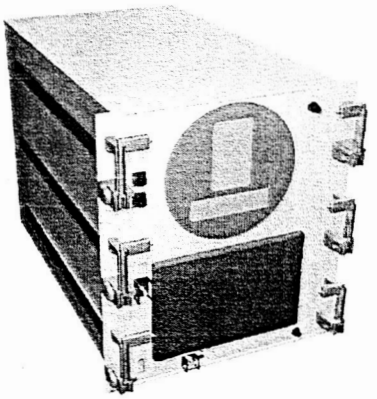
3-D model of SEU



3-D model of Flight Unit



SEU configured for test with C2C12 muscle cells



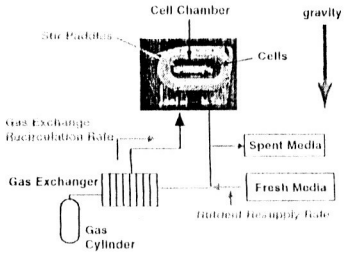
External view of flight configuration

Unique Capabilities of the Cell Culture Unit – Good Opportunity for Early Science:

Cell Culture Unit (CCU) is uniquely designed to independently control mixing via stir bars, gas exchange via the recirculation flow rate, and the nutrient/spent nutrient exchange via the nutrient resupply rate

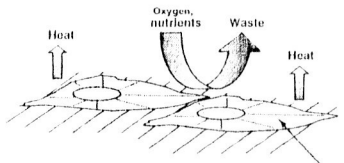
The CCU operates as a perfusion based system, that also has stir bars to allow mixing within the chamber. Independent control of these parameters should enable experiments to separate the effect of gravity as a body force acting directly on cell structure vs. the effect of gravity acting to alter the mass transport environment around the cell.

CCU design - Independent control of:



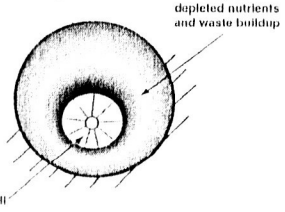
Normal gravity

convection drives exchange of nutrients and waste



Microgravity

No gravity = No convection
Nutrient exchange is diffusion limited



CCU automated functions - *Experiments can be run without crew intervention.*

- Automated sampling module that is temperature controlled from 39 to 4 degrees C
- Self-contained video microscopy
- Automated subculture
- Media and gas exchange
- Automated additive addition
- Fixation of samples withdrawn from chamber as well as washing and fixation of the cells inside the chamber

Rigorous ground testing of the hardware with seven cell types is underway.

The CCU is unique in the extent and type of ground testing performed during its development. The cell chambers are being tested for both adherent and suspension cultures by both the developers and by academic investigators that are independently assessing the hardware.

CCU has a video microscopy system that views all chambers on a rotating basis.

There is no need for removal of cells to the glovebox for basic microscopic analysis. Both adherent and suspension cultures can be viewed with a magnification selected preflight (40X-200X) using differential interference contrast (DIC) or brightfield imaging techniques.

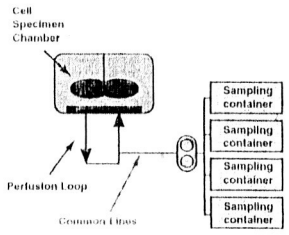
CCU supports adherent cells on an optically clear substrate that allows for visualization of cell morphology.

CCU can seed suspension cells on orbit without crew intervention.

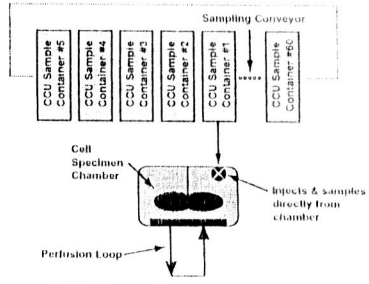
CCU can seed yeast from dried powder from the automated sampling module into the chambers and this action can be controlled by the investigator on the ground.

CCU has media sampling and cell sampling direct from the cell chamber.

There is no contamination from previous samples as is the case in other hardware in which samples are taken from a common line.



Typical Sampling Approach:
Common Line to Sample Containers = Cross Contamination Between Samples



CCU Sampling Approach:
No Common Lines = Pure Samples

CCU has the capability to wash the cells before and after fixation.

This is a standard laboratory practice that is not offered by other hardware and should result in better fixation and therefore better studies of the effects of space flight on cellular morphology.

Placement of the CCU on the long arm of the ISS centrifuge decreases the inertial shear stress cells experience when placed on smaller centrifuges and allows for a much more uniform g value across samples in the CCU.

The CCU is the only cell culture habitat designed to work on the ISS centrifuge.

CCU offers greater number of cell chambers and sampling capacity in combination with long duration experiments.

The CCU offers up to 18 cell specimen chambers and 60 individual sample containers. Investigators are concerned about having a sufficient number of individual cell cultures, maximizing the "n" of a given experiment, and this philosophy is incorporated into the CCU design. The CCU cell chambers and sample containers can be changed out on orbit, to produce a high "n" and accommodate long duration experiments.

What CCU does require from crew time if performing long duration experiments:

- Change out of media bags, new and spent.
- Change out of sampling containers:
  There are up to 60 containers per CCU, and samples can be maintained at 4 °C, but in a 90 day period, more may be required, or some samples may need to be transferred to a freezer for better storage.
- Change out of cell chambers:
  Up to 18 4ml chambers are in each CCU, but in long multi generational experiments, this number may be too few, and the crew can access the chambers and replace them with fresh chambers as needed.

Cell Culture Unit Flow Environment Characterization

Approach

Collaborations with the hardware developer and multiple NASA Centers have been formed to characterize and optimize the fluid flow environment inside the Cell Culture Unit (CCU) Cell Specimen Chamber (CSC).

- NASA Ames
- NASA Glenn
- Payload Systems, Inc.

Goals

Provide rapid mixing and cell suspension

Minimize time and fluid volume required to flush cell growth chamber

Minimize forces on cells.

Ensure functionality from μ-g to 2-g

Techniques

Dye Front Flow visualization

Particle Imaging Velocimetry (PIV)

Computational Fluid Dynamics (CFD)

Centrifugation Experiments

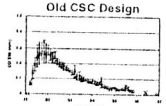
Mixing, Suspension and Flushing

Uniformity of nutrient/oxygen supply

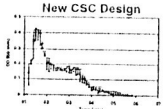
Flow rate [mL/min]	Stirring speed [rpm]	Chamber mixing time [s]
0.83	0	240
0.83	40	3

Suspension Results with Cells

Flushing Efficiency: Flushing performance of water into a dye filled Cell Specimen Chamber (CSC)



95% flushed after 55 min/45.7 ml



95% flushed after 40 min/33.2 ml

Chamber redesign yielded 27% savings in volume required for a 95% complete flush

Dye in CSC (new design)

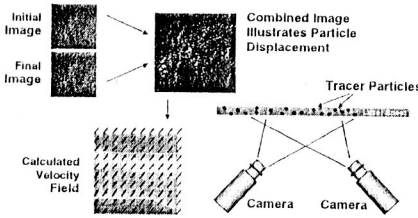
Flushed 10 min 20 sec
8.5 ml used (new design)

Flushed in 28 min 55 sec
24 ml used (new design)

Forces on Cells, 3-D PIV, PIV Test Setup

3-D Particle Image Velocimetry (PIV)

- Measures 3-D velocities in a plane (1-4% Errors)
- 2-Camera stereoscopic viewing



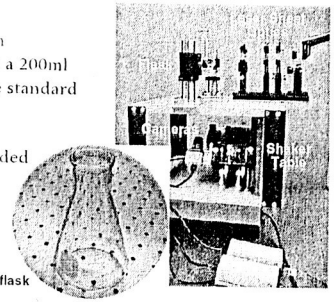
PIV Testing Setup / Plans

Shaker Flask Testing at 120 rpm

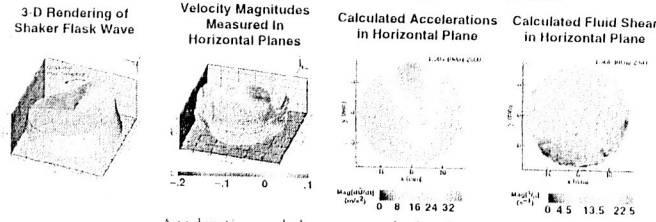
- Determine forces on cells in a 200ml flask on a shaker table, the standard lab approach

CSC Unit Testing

- Determine forces on suspended particles in CSC
- Compare to results from shaker flask testing



Particle Imaging Velocimetry (PIV) Results: Testing of Shaker Flask



Acceleration and shear were calculated from measured velocities

Maximum Acceleration 70 m/s^2

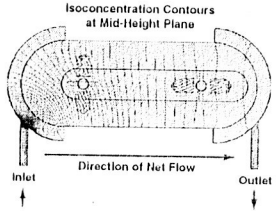
Maximum Shear 3.74×10^{-4} N/m^2

Computational Fluid Dynamic Analysis

CFD provides a tool to analyze the fluid environment in the chamber, including the concentration fields, shear stresses and other forces that act on the cells in the chamber.

CFD can be used in a predictive capacity to specify stirring and perfusion rates that supply cells with an optimal environment.

Velocity vectors colored by velocity magnitude

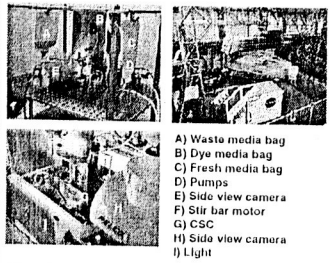


CFD analysis will allow rapid prediction of:

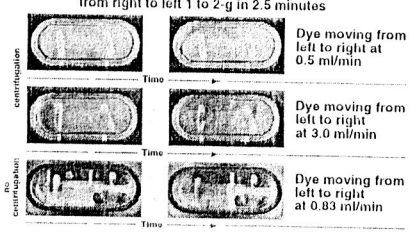
- Three-dimensional flow
- Effects of gravity changes on CSC performance
- Flow changes due to CSC redesign and pump scheme optimization
- Forces acting on cells in culture
- Mass transfer characteristics

Centrifugation Experiments

HyFaCC Experiment Hardware



Dye Front Flow Visualization Results



Use the Hypergravity Facility for Cell Culture (HyFaCC) for Dye Front Flow Visualization and cell culture experiments

- Single arm centrifuge, nine foot radius
- Incubator mounted on swing bucket at end of centrifuge arm
- Single loops mounted on incubator shelf

Objectives:

- Characterize the flow properties at 2-g using flow visualization with dyes
- Study effect of ramp-up on dye front using ISS Centrifuge Rotor spin-up rates
- Study suspension and membrane fouling characteristics using cells

Additional Benefits:

- Facility available to Principle Investigators for future 2-g cell experiments

Conclusions

Dye Front Flow Visualization

- Coriolis Effect from centrifugation has a significant impact on CSC flow field characteristics. The effect is present at all times during centrifugation, but is more pronounced during acceleration and at higher centrifuge rpm.
- Without the use of stir bars, the centrifugation affected flow fields could impact any cell culture science.

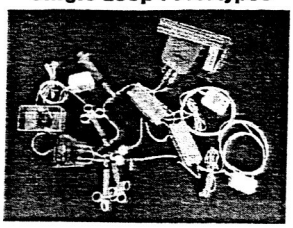
BY-2 Tobacco Experiments

- The hardware used for CCU Single Loops functioned properly in a hyper-gravity environment
- A 2-g environment required a 50% increase in stir bar rpm
- Gross cellular morphology and viability were not effected by 6-8 hour centrifugation at the increased stirring rate

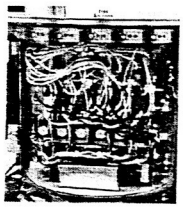
Cell Culture Unit Development Philosophy

- Develop and test Science Evaluation Unit (SEU) prior to Critical Design Review (CDR) to evaluate effectiveness of the Cell Culture Unit (CCU) design to support the required cell experiments.

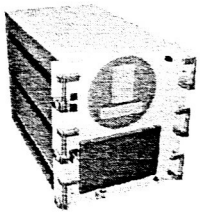
Single Loop Prototypes → **SEU** → **CCU**



Basic perfusion loop with flight-like cell chamber

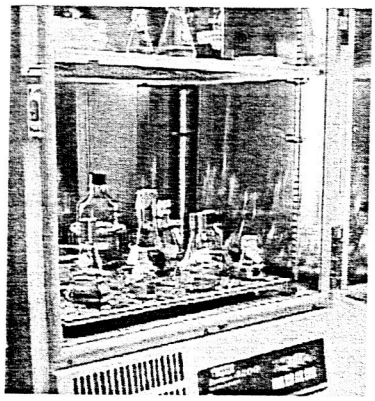


Development hardware used to test cell growth in flight-like environment

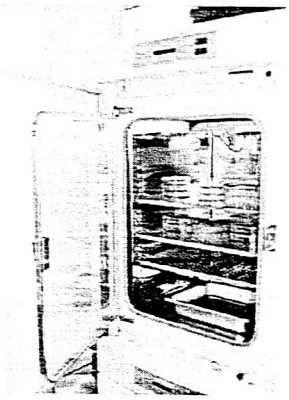


Flight Unit

- Define what a cell needs to grow
  - Nutrients & regulatory molecules from culture medium (pH 5.6 - 7.4)
  - Temperature Control (15 - 37°C)
  - Gas Exchange
  - Cell attachment surface or means of stirring to maintain suspension

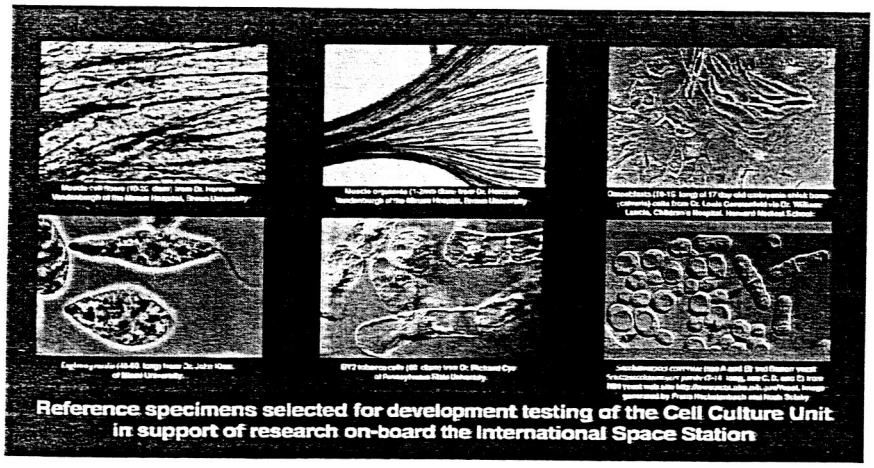


Suspension cell cultures grown in shake flasks in incubator



Adherent cell cultures grown in tissue culture vessels in incubator

- Define scientifically important cell types that challenge hardware functionality



Reference specimens selected for development testing of the Cell Culture Unit in support of research on-board the International Space Station

- Optimize experiment conditions in simple single loop hardware.
- Analyze and test flow environment to support SEU development and testing.
- Perform SEU experiments at PSI.
- Perform SEU experiments independently at ARC.

Cell Growth in the Cell Culture Unit: Recent Test Results

YEAST, *Saccharomyces cerevisiae*

These results are from previous tests at Payload Systems Inc. Similar results were obtained from the CCU-SEU test held at ARC August 13-17, 2001.

BACKGROUND

- Yeast are a single celled organism that have an indisputably high value to science. Broadly used as a model organism across disciplines, yeast biology is particularly powerful in the pursuit of gene expression changes caused by space flight.
- Environmental stresses, such as space flight, elicit responses in yeast that are highly conserved in evolution, therefore information gained from studies of yeast are relevant to higher organisms, including humans.

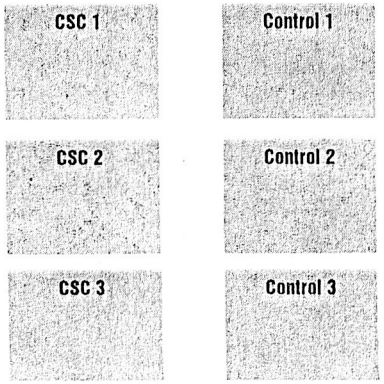
TEST DESIGN

- Yeast were cultured in the SEU and in control shaker flasks for four subcultures.
- Cell count, pH, Oxygen concentration, morphology, absence of contamination, and maintenance of genotype were assayed.

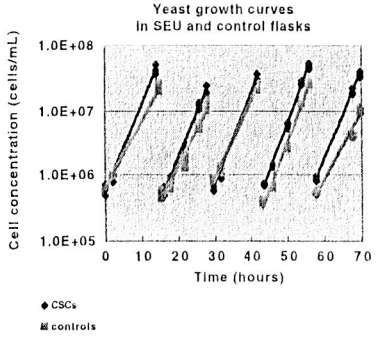
RESULTS

- The SEU performed as expected.
- Cell growth and viability of cultures in the SEU were comparable to control flask cultures.
- Cell morphology was comparable between SEU and control cultures.
- pH and oxygen concentration of the yeast media were similar to control flasks.

Yeast grown in SEU and control flask have Comparable Morphologies

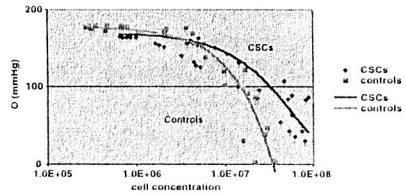


Yeast growth rate in SEU is comparable to control flask

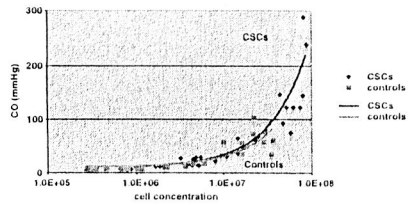


Yeast: gas levels

Oxygen concentration better maintained in CSCs than in control flasks



Carbon dioxide concentration comparable in CSCs and control flasks



C2C12 muscle cells

These results are from tests performed using single loop hardware at Payload Systems Inc.

BACKGROUND

- C2C12 cells are undifferentiated murine skeletal myoblast cell line derived from mouse leg muscle that differentiate into muscle fibers in culture.
- Because of the muscle deterioration seen in astronauts following extended spaceflight, muscle cell biology is of great interest to the gravitational biology community.

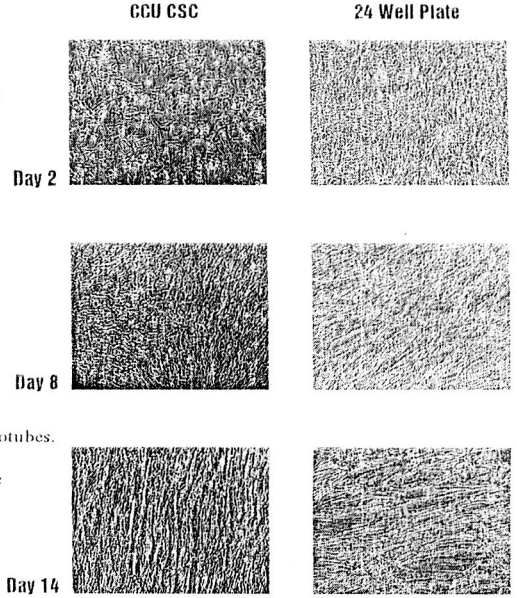
TEST DESIGN

- Cells attach and grow in monolayer to confluence.
- Grown on tissue culture plastic (control), and on entactin/ collagen/ laminin or Matrigel™-coated glass in CCU testing.
- Confluent cells differentiate to form myotubes.
- The myotubes are then stained with antibodies to tropomyosin to determine if differentiation occurred.

RESULTS

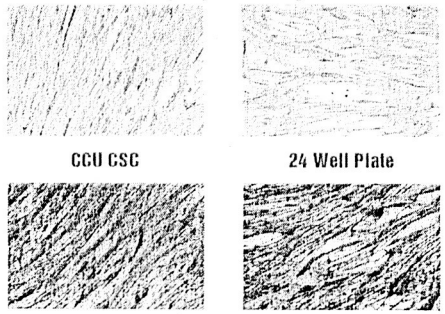
- Cells are viable, grow to confluency and differentiate.
- Myotube formation is slightly delayed in cells grown in CCU hardware as compared to controls.
- Tropomyosin expression is similar between CCU and control cultures.

C2C12 muscle cell growth and differentiation: CCU hardware vs. tissue culture plate control



C2C12 muscle cells at day 14 stained for the muscle fiber differentiation marker tropomyosin.

Tropomysin Staining



Tropomysin Stainingwith Hematoxylin Counterstain

Other cell types grown in CCU prototype hardware

Euglena gracilis - Motile, non-feeding aquatic organism*

Muscle organoid*

Tobacco BY-2 cells

Human primary dermal Fibroblasts

Human primary osteoblast - bone cells

*Successfully grown in single loop hardware at Payload Systems Inc. and at independent consultants.