Biology in Space: past, present and future of biological experiments onboard ISS supported by ESA

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1. Introduction: the ISS as a Lab
2. How to run an experiment onboard ISS: facilities and constraints
3. Cell biology experiments in Kubik and Biolab
4. Plant biology experiments in the EMCS
5. Life support system and related experiments
6. Conclusions
How the ISS looks like?
# The ISS is very different from a terrestrial laboratory environment

<table>
<thead>
<tr>
<th>Earth based Biology Lab</th>
<th>ISS Biology Lab</th>
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<tbody>
<tr>
<td>Perform a wide research program</td>
<td>Typically one flight experiment per project</td>
</tr>
<tr>
<td>Multiple experiments, experimental program adapted according to results</td>
<td>Experiment specific hardware developed according to experiment requirements</td>
</tr>
<tr>
<td>Use off-the-shelf equipment and consumables</td>
<td>Experiment operations constrained by capabilities of ISS facilities as well as other ISS operations and experiments</td>
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<td>Science team performs several experiments in a sequence in order of hours - weeks</td>
<td>Many entities involved in definition, development, implementation and operation of ISS experiments</td>
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<td></td>
<td>Lead time to perform experiment between 18 months – 5 years</td>
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ELIPS - European Programme for Life and Physical Sciences in Space

- ELIPS is since 2002 ESA’s key programme to conduct research on ISS
- To augment efficiency and impact of ISS experiments, also using other platforms
- Support Industrial applications and involvement
**Focus of biological research:**

- **Plant Biology:** Study of the growth factors (gravity, light)
- **Developmental Biology:** organisms / ecosystems for biological Environmental Control and Life Support Systems. Development / evolution of living things
- **Cell and Molecular Biology:** Biotechnology experiments (cells, tissues, bacterial cultures) for potential applications in medicine, agriculture, environment.
A Biology Experiment onboard ISS has usually specific and unique requirements which differ from those on ground.
Key steps to a fly a Biological experiment on ISS

1. Selected Experiment Proposal
2. Science Requirements Definition
3. Baseline Science Requirements
4. Define Experiment Hardware Requirements
5. System Requirements Document (SRD)
6. Experiment Scientific Requirements (ESR)
7. Experiment Hardware Development, Qualification and Acceptance
8. Implementation and Operational Plan & Documentation
9. ISS Operational constraints
10. Pre-launch preparation campaign
11. Experiment Execution Onboard ISS
12. Postflight Analysis of Data

Update if necessary
Updates reviewed & Agreed with all parties
Typical cell biology experiment on ISS

On ground preparation → H/O → Launch & Upload → Installation (Kubik/Biolab)

Fixation ← Experiment

Storage ← Download → Early retrieval to PI

1g

Micro-g
Constraints

On ground preparation → H/O → Launch & Upload → Installation (Kubik/Biolab)

Time? Temperature?

Fixation ← Experiment

Storage ← Download ← Early retrieval to PI
Constraints

- On ground preparation
- H/O
- Launch & Upload
- Installation (Kubik/Biolab)

G-level? Temperature?

- Fixation
- Experiment
- Storage
- Download
- Early retrieval to PI

European Space Agency
Constraints

- On ground preparation
- H/O
- Launch & Upload
- Installation (Kubik/Biolab)

- Fixation
- Storage
- Download
- Early retrieval to PI

Experiment

Duration? Temperature? Medium refresh? Gas exchange?
Constraints

On ground preparation → H/O → Launch & Upload → Installation (Kubik/Biolab)

PBS wash? Fixative? Duration?

Fixation → Experiment

Storage → Download → Early retrieval to PI

European Space Agency
Constraints

- On ground preparation
- H/O
- Launch & Upload
- Installation (Kubik/Biolab)
- Fixation
- Experiment
- Storage
- Download
- Early retrieval to PI

Duration? Temperature?
http://www.nasa.gov/mission_pages/station/research/experiments/GLACIER.html
The challenge is to adapt a normal and usual lab protocol to the whole ISS experiment, taking into account all the constraints in terms of requirements.

The keyword is **TESTING**
Typical key requirements

Volumes and sizes

Volume is one of the main drivers for selecting the servicing facility:

- **BIOLAB:**
  - Standard EC: 100 x 60 x 60 mm (6 ECs)
  - AEC: 108 x 150 x 137 mm (2 Ecs)

- **KUBIK**
  - Type I EC: 81 x 40 x 20 mm (16ECs in micro-g and 8ECs in the centrifuge)

The limited volume availability can affect cell viability.

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Typical key requirements

**Time and temperature**

- Often inter-related
- Specific to individual components (e.g. cells, medium, fixative)
- Time & temperatures can greatly vary during the different steps/phases of the experiment (upload, execution of the experiment onboard, storage of samples, download, early retrieval) due to specific constraints
Number and type of samples

• Preferred and minimum needed
• Linked to the selected facility (Kubik, Biolab)
• Number of replicates needed
Example: Kubik can host up to 16 ECs in micro-g and 8 ECs in the centrifuge
Typical key requirements

**Toxicity level**

- Chemicals for fixation
- Level of containment
- Any alternative? For example: NOTOXhisto
Typical key requirements

Gas requirements / gas exchange

- Oxygen, CO2, trace gases (e.g. Ethylene)
- CO2-dependent medium
- CO2-independent medium: keeping a stable pH under normal CO2 atmospheric concentrations
Basically there are 4 choices:

1) Kubik
2) EMCS
3) Biolab
4) Stand-alone
1. Kubik is a **small controlled-temperature incubator** or cooler used to study biological samples in a microgravity environment.

2. Kubik is a **cubic box** container measuring 37 cm by 37 cm by 37 cm composed of (from top to bottom) a thermal chamber (26 cm by 26 cm by 12.8 cm), thermal block with Peltiers (heat pumps with a hot side and a cold side) including exchangers and fans, and electronic boxes used to control the incubator and inserts.

3. There are **no data or command communication** possibilities between the experiments and Kubik.
1. Kubik operates from \textbf{6°C to 38°C}.

2. ESA usually develops \textbf{experiment-specific hardware}, also known as the Experiment Unit (EU), according to the requirements of the experiment.

3. \textbf{Centrifuge Insert (CI)}: The CI has a settable acceleration between 0.2g to 2g in 0.1g increments. It accommodates either 16 standard size containers in static positions or 8 standard size containers and 4 extended containers; the CI also accommodates 8 standard size or 8 extended containers.
The spaceflight experiment, renamed “SPHINX - SPaceflight of Huvec: an INtegrated eXperiment”, was presented to ESA in the frame of ELIPS Programme in September 2009. SPHINX has been selected and scheduled for flight on Progress 40P (27 October 2010).

**HYPOTHESIS**
How HUVECs modify their behaviour when exposed to real microgravity. This could provide better knowledge of endothelial function, which could be useful for clinical application.
RESEARCH OBJECTIVES

The specific objectives of the SPHINX experiment are the evaluation of endothelial:

• *protein profile by protein arrays.*
• *gene expression by cDNA arrays.*
• *synthesis of nitric oxide (NO).*

as target analysis for evaluating endothelial disfunction
The FASEB Journal article f.13-229185. Published online August 2, 2013.

The FASEB Journal • Research Communication

The challenging environment on board the International Space Station affects endothelial cell function by triggering oxidative stress through thioredoxin interacting protein overexpression: the ESA-SPHINX experiment

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ABSTRACT Exposure to microgravity generates alterations that are similar to those involved in age-endothelial behavior, and promote senescence—Veronci, S., Longinotti, G., Baronghi, L., Maier, J. A. M.,
L = Launch
D = Docking
T₀ = installation in KUBiK
A = 1st medium exchange
B = 2nd medium exchange
C = 3rd medium exchange

Time (h): 24, 96, 144, 192, 240

Temperature (°C):
- Start: 37°
- 96h: 36.5°
- End: 6°
TABLE 3. RT-PCR of a selection of genes to validate the microarray results

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene assignment</th>
<th>Fold change array</th>
<th>Fold change RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TXNIP</td>
<td>Thioredoxin-interacting protein</td>
<td>10.5</td>
<td>33.0</td>
</tr>
<tr>
<td>MIR15A</td>
<td>MicroRNA 15a</td>
<td>2.6</td>
<td>5.0</td>
</tr>
<tr>
<td>TP53INP1</td>
<td>Tumor protein p53-inducible nuclear protein 1</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>HSPA1B</td>
<td>Heat-shock 70-kDa protein 1B</td>
<td>−3.7</td>
<td>−5.6</td>
</tr>
<tr>
<td>HSPA1A</td>
<td>Heat-shock 70-kDa protein 1A</td>
<td>−2.6</td>
<td>−5.6</td>
</tr>
<tr>
<td>CLCA2</td>
<td>Chloride channel accessory 2</td>
<td>−2.3</td>
<td>−4.1</td>
</tr>
<tr>
<td>EDN1</td>
<td>Endothelin 1</td>
<td>−1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>IL1A</td>
<td>Interleukin 1, α</td>
<td>1.0</td>
<td>12.6</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>−1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>NOS2</td>
<td>Nitric oxide synthase 2, inducible</td>
<td>−1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>NOS3</td>
<td>Nitric oxide synthase 3 (endothelial cell)</td>
<td>−1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
<td>−1.9*</td>
<td>0.9</td>
</tr>
</tbody>
</table>

RT-PCR was performed in triplicate using the same cDNA preparations as those used for the microarray analysis. Mean fold induction or repression of spaceflight vs. 1-g control samples. Fold change values of the microarray data are included for comparison. *Fold change within the cutoff value but $P > 0.01$. 
1. The EMCS is an ESA gravitational biology payload and installed inside the **US Destiny laboratory**.

2. EMCS is mainly dedicated to experiments on **plants**, especially multi-generation (seed-to-seed) experiments and studies gravity effects on early development and growth, on signal perception and transduction in plant tropisms.

3. EMCS consists of a gas tight **incubator** containing **two centrifuges** with space for 4 Experiment Containers on each rotor; also the **life support and water supply system**, the **illumination** and the **observation** system are located on the rotors.
HYPOTHESIS
Amyloplasts displacement triggers calcium signalling cascades in roots. GRAVI-2 experiment will help to better understand root gravisensing and cellular signalling mechanisms involved during a threshold acceleration.

OBJECTIVES
GRAVI-1: threshold acceleration for root gravisensing (Driers-Ecole et al., 2008). GRAVI-2: further investigate the gravitational behaviour of lentils (Lens culinaris) seedlings and response after various microgravity growth periods followed by low and short high level of gstimulations. The calcium localisation, calcium-binding proteins and calcium-dependent signalling pathways will be, in particular, investigated and are linked to distribution of amyloplasts
From Gravi-2 experiments:

- Amyloplast displacement?
- Calcium-signalling pathways?

- 21 h μg + 2 g for 5 min
- 21 h μg + 2 g for 15 min

- Important amyloplast displacement toward the membrane
- Succession of signalling pathways

Space control
Seedling Growth

Joint experiment ESA-NASA with hardware developed by NASA and use of the ESA-EMCS

SG-1 under NASA responsibility
SG-2 under ESA/NASA responsibility
SG-3 under ESA responsibility
Seedling Growth

Cellular Level:
- Growth Coordinators (Meristematic Competence)
  - Cell Growth
    - Ribosome Biogenesis
    - Protein Synthesis
  - Cell Proliferation
    - Cell Division Cycle
    - DNA Synthesis

Gravity & Light
- Auxin Distribution in Roots
- Root Growth & Development

Gravitropism
- Gravimorphism

Phototropism
- Few evidences of direct relation with cellular events
Biolab (Biological Experiment Laboratory) is a single-rack multi-user science payload designed for use in the Columbus.

Biolab support biological research on small plants, small invertebrates, microorganisms, animal cells, and tissue cultures. It includes an incubator equipped with centrifuges in which the preceding experimental subjects can be subjected to controlled levels of accelerations.
The BioLab facility is divided into two sections: the **automated section** and the **manual section**, designed for crew interaction with the experiments.

The **A.S.**, which can operate autonomously or telerobotically (via commands sent from the ground), consists of a large **incubator**, two **centrifuges**, a **microscope**, a **spectrophotometer** (an instrument used to measure the spectrum of light absorbed by a sample), a sample-handling mechanism.

The **M.S.** consists of the Experiment Preparation Unit, the **BioGloveBox**, and additional **Temperature Control Units** for storing experiment containers (ECs) and preserving samples.
BioLab's **microscope**, which can be controlled by investigators on the ground, has a resolution that ranges from 0.6 to 1.8 micrometers (µm) with a 0.25 µm and 1.0 µm diameter field of view, respectively.

The **spectrophotometer**, which uses tungsten and deuterium lamps, can analyze light passed through the sample in the spectral range of 220 to 900 nm (ultraviolet, visible, and near infrared) with a resolution of 10 nm.
An example of experiment into the Biolab: TRIPLELUX

Main Features:
- Cell cultivation between 18 and 37°C, at μg or 1g
- Luminescence measurement (photon counting) at 350 to 550 nm for several hrs
- Data downlink
- 1 or 2 cultivation cuvettes
- 4x measurement cuvettes
- 1 or 2 liquid reservoirs for automatic injection
- Gas supply via gas permeable material
- Biocompatible
- Irradiation of cell culture by integrated UV-LED (Part C, optional)
- Density measurement (optional)
- Magnetic stirrer to avoid sedimentation on the 1g centrifuge
- Automatic liquid distribution inside the experiment container
- Return of biological samples at low temperature possible

Experiment Part A & B:
Observation of the rate of phagocytosis in vertebrate (A) and invertebrate (B) immune cells to investigate the effects of microgravity and LEO cosmic radiation on this basic immune function.
The ability of leukocytes to phagocytose zymosan (as an analogue of bacteria) will be assessed. This process is the first line of defense against microbial infection. Phagocytosis will be quantified using **luminol** as a detector for reactive oxygen species produced during phagocytosis of **zymosan**.
Oxidative burst

NADPH-oxidase produces $O_2^-$
The reactive oxygen burst is measured by a chemiluminescent assay which where \( \text{O}_2^- \) radicals convert luminol to 3-Aminophthalate which results in the emission of light at 475nm, this is illustrated below. Light emission is enhanced by the additional of exogenous hydrogen peroxide.
Life support system
**MELiSSA** (Micro-Ecological Life Support System Alternative) has been conceived as a micro-organisms and higher plants based ecosystem intended as a tool to gain understanding of the behaviour of artificial ecosystems, and for the development of the technology for a future regenerative life support system for long term manned space missions - for example: a lunar base or a mission to Mars.
The cyanobacterium *Arthospira* sp. strain PCC8005 is a candidate for use in spacecraft biological life support systems, for CO₂ and nitrate removal, and oxygen and biomass production. However, to ensure the reliability of such a biological life support system it is necessary to **characterize the response of *Arthospira* sp. PCC8005 to in situ spaceflight conditions.**

Examine the **response** of *Arthospira* sp. strain PCC8005 to spaceflight conditions at the culture level (cell density, cell interactions), cellular level (size, shape, colour, membrane structure), and molecular level (metabolomic, lipidomic, proteomic, transcriptomic and genetic level).

Determine the **kinetic parameters** for subsequent mathematical modelling of *Arthospira* sp. strain PCC8005 reproduction and metabolism under spaceflight conditions.
Conclusions

1. ISS as an unique Lab for biology experiments
2. Constraints make an experiment challenging
3. The success of an experiment on the ISS is the result of a tight interaction between several ESA entities
4. The ISS permits to achieve significant scientific results
THANKS FOR YOUR ATTENTION