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Cells in Space:
a journey through simulated microgravity

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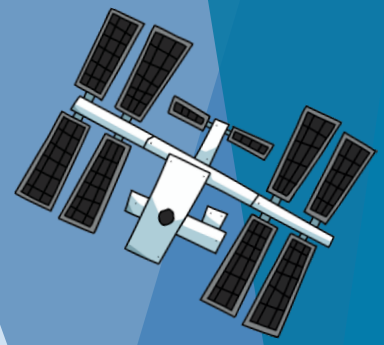
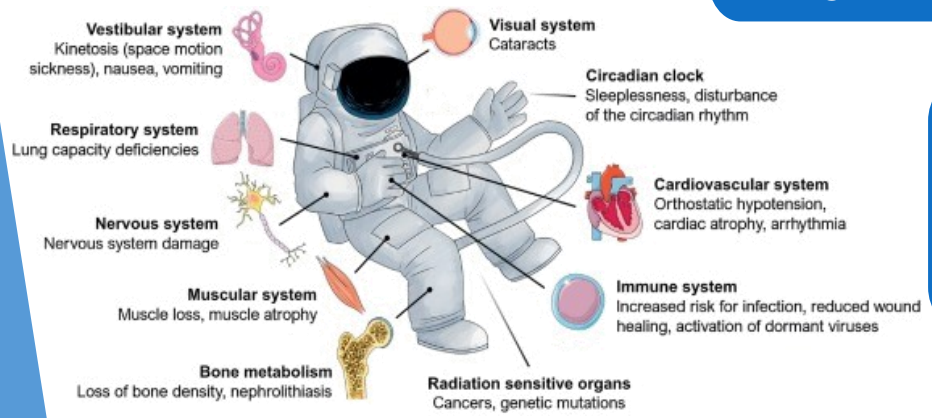
Background



The increasing number of spaceflights and the permanence of astronauts in orbit to maintain satellites and space stations have focused attention on the effects induced by altered gravitation on the human body.

Microgravity affects numerous features and functions of biological organisms.

Macroscopic evidences of the whole body can often be the result of modifications at tissue and cellular levels. The study of the mechanisms that underlie the effects induced by microgravity in space are made difficult by limitations due to logistic difficulties and in the gathering of enough material from astronauts.



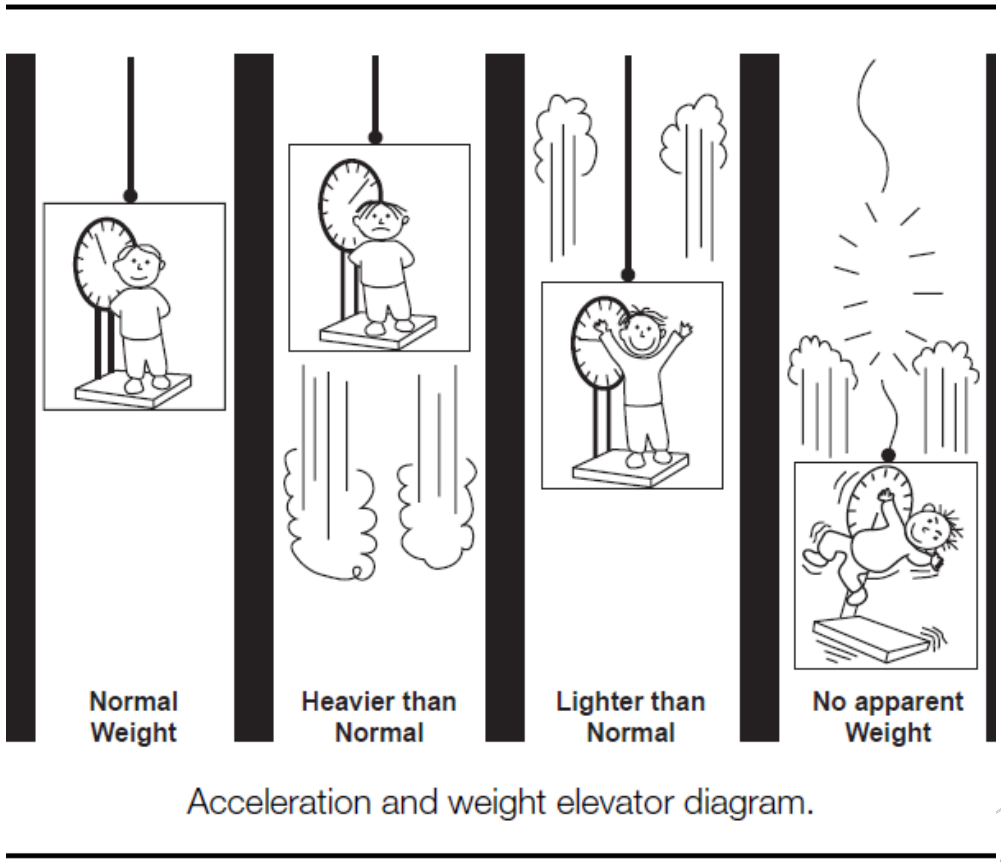
The characterization of the mechanisms triggered by microgravity exposure have required the development and improvement of models of simulated weightless environment, also to search for protective strategies.

Moroni L et al. Trends in Biotechnology 2022

Definitions

- **Zero gravity-Microgravity**. Many people use the words interchangeably, but there is a difference, and to astronauts and scientists, the difference is significant.

- The term **microgravity** is used to describe a condition where gravity is not small, but appears to be small. This occurs on an orbiting spacecraft, such as the International Space Station (ISS), and all objects in free-fall.



Acceleration and weight elevator diagram.

Educational Brief NASA's Bioreactor: Growing Cells in a Microgravity Environment , 2002

The technical approach to simulate the microgravity in the lab at cellular levels

The experimental models

- Experimental phases: ***in vitro***, ***ex vivo***, ***in vivo***
  - Choice of biological models
    - Choice of instrumentation

- **Interdisciplinary interaction**

- **Results:** analysis and interpretation
  formulation of theoretical models

The experimental model

- Advantages: a controlled system

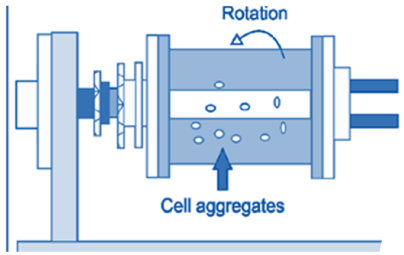
- Disadvantages: too much simplified

- For each simulator, the physical parameters and principles as well as their specific impact on the biological processes and objects of different sizes need to be critically evaluated.

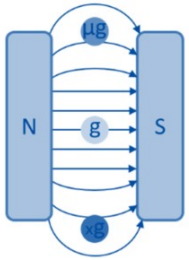
The instrumentation

- **Variability** - Numerous experiments have been performed with different types of simulators and a great variety of organisms
- **Results** - Simulator experiments have provided excellent insights into a multitude of gravity-dependent phenomena
- **Limits** - Many results from experiments in simulated microgravity was not reproduced in real microgravity conditions
- **Warning** - Non-critical use of simulators may easily result in a misinterpretation of responses to side effects as specific microgravity effects

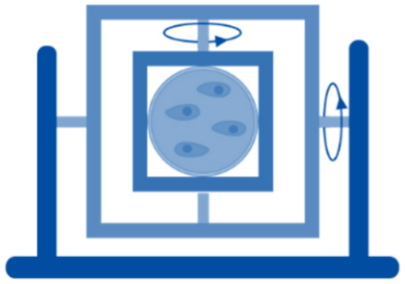
Herranz et al, Astrobilogy 13:1, 2013



Instruments for in vitro experiments

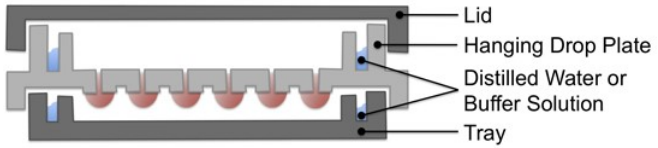


- For simulated microgravity: all tools that create a controlled environment in which the gravity is countered

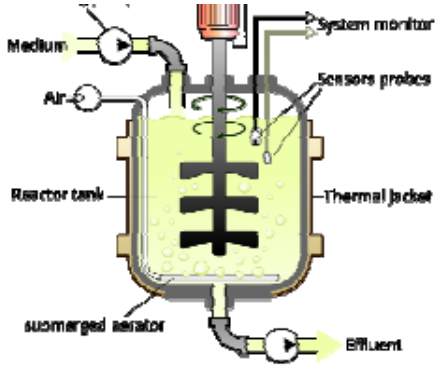


From the simplest……

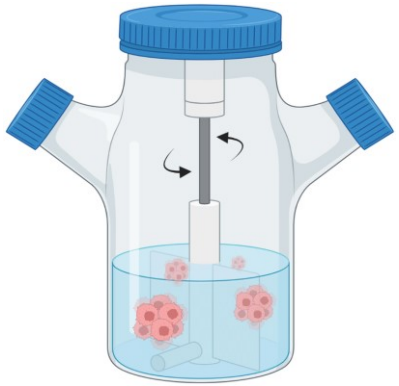
- Hanging drops of cell cultures



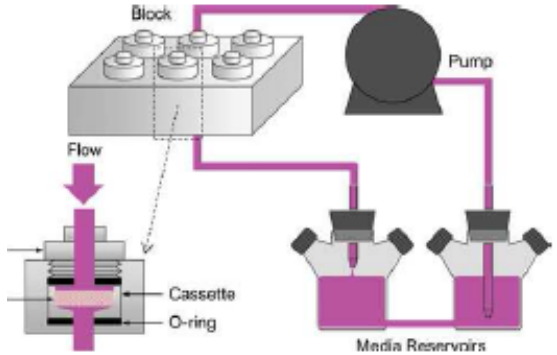
- Batch cultures



- Spinner flask

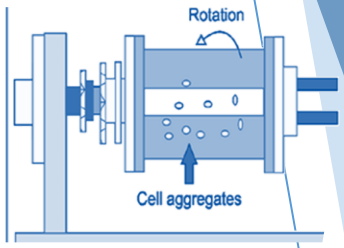


- Fed-perfused cultures

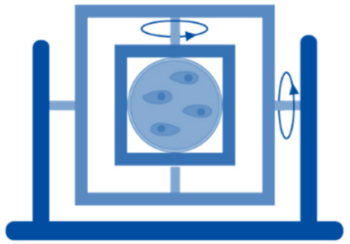


The most used microgravity simulators

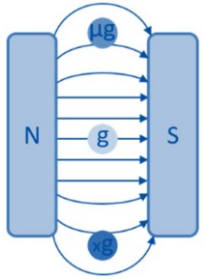
- 2-D clinostat (e.g. Rotating Wall Vessel)



- 3-D clinostat (Random Positiong machine)



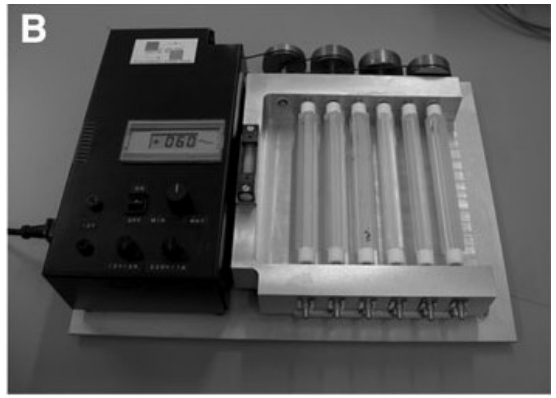
- Diamagnetic levitation



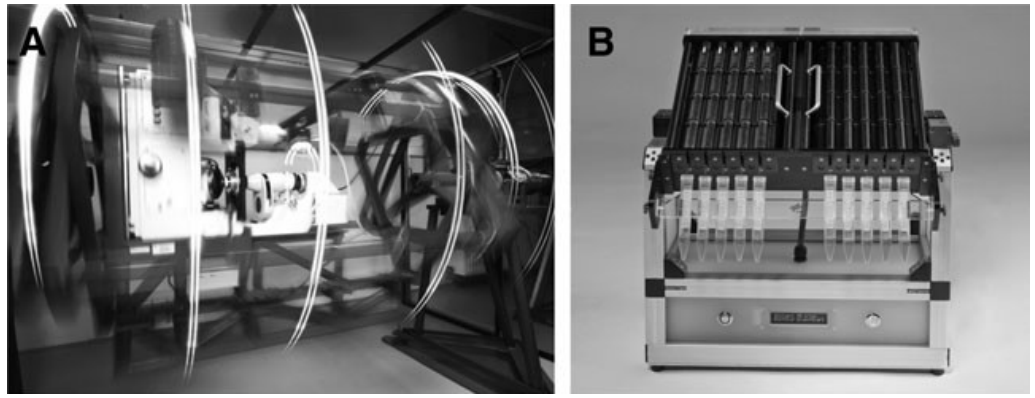
Herranz et al, Astrobilogy 13:1, 2013

2-D clinostat

▶Devices suitable for tissue engineering. (A) Two-dimensional clinostat in an incubator constructed by the German Aerospace Center (DLR), Institute of Aerospace Medicine, Biomedical Science Support Center, Gravitational Biology, Cologne, Germany. (B) Example of a fast-rotating 2D clinostat manufactured by CCM (Neunen, The Netherlands). The system holds three static and three rotating tubes of about 10mL volume. Rotation speed can be adjusted between 30 and 150 rpm. (Image: J. van Loon, DESC, Amsterdam, The Netherlands) (Grimm et al, Tissue Engeenering 2014).



2-D clinostat



Images of the 2-D clinostat microscope (A) and the pipette 2-D clinostat (B) hosted at DLR, Cologne, Germany (Herranz et al, Astrobiology, 2013)

Considerations: The tubes have an **internal diameter and a rotation speed in relation to the biological sample**. The use of clinostats in plant research began with experiments that rotated the object relatively slowly (1–10 rpm; classical clinostat). **Seedlings and small plants rotated slowly in the 2-D clinostat axis did not exhibit any gravitropic response, but showed disturbances at the ultrastructural level, which were not found under spaceflight conditions (Hensel and Sievers, 1980). These are indications that the slow rotation prevented a gravity-induced growth response but most likely also caused omnilateral mechanical stress in some sensitive plant tissues.**

It is argued that the coupling between the cells and their respective static surrounding liquid boundary layers is the main reason for this microgravity simulation paradigm. **A balance between fluid density, viscosity, and cell-specific density is necessary.**

2-D clinostat: Rotating wall vessel (RWV)

Culture vessel

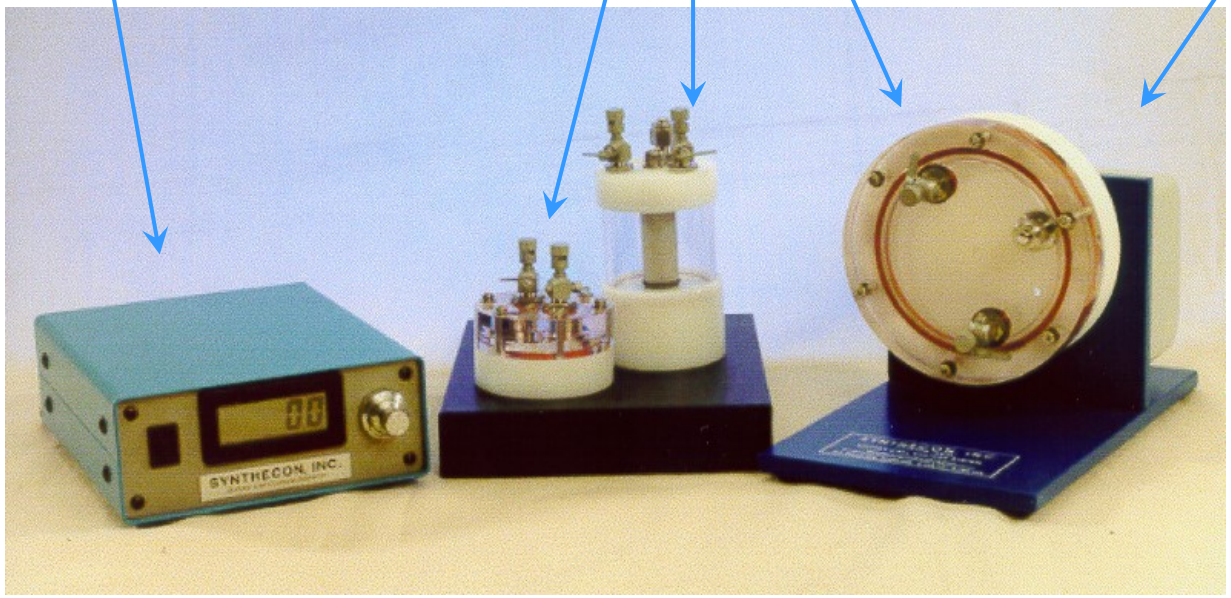
different sizes

Control unit

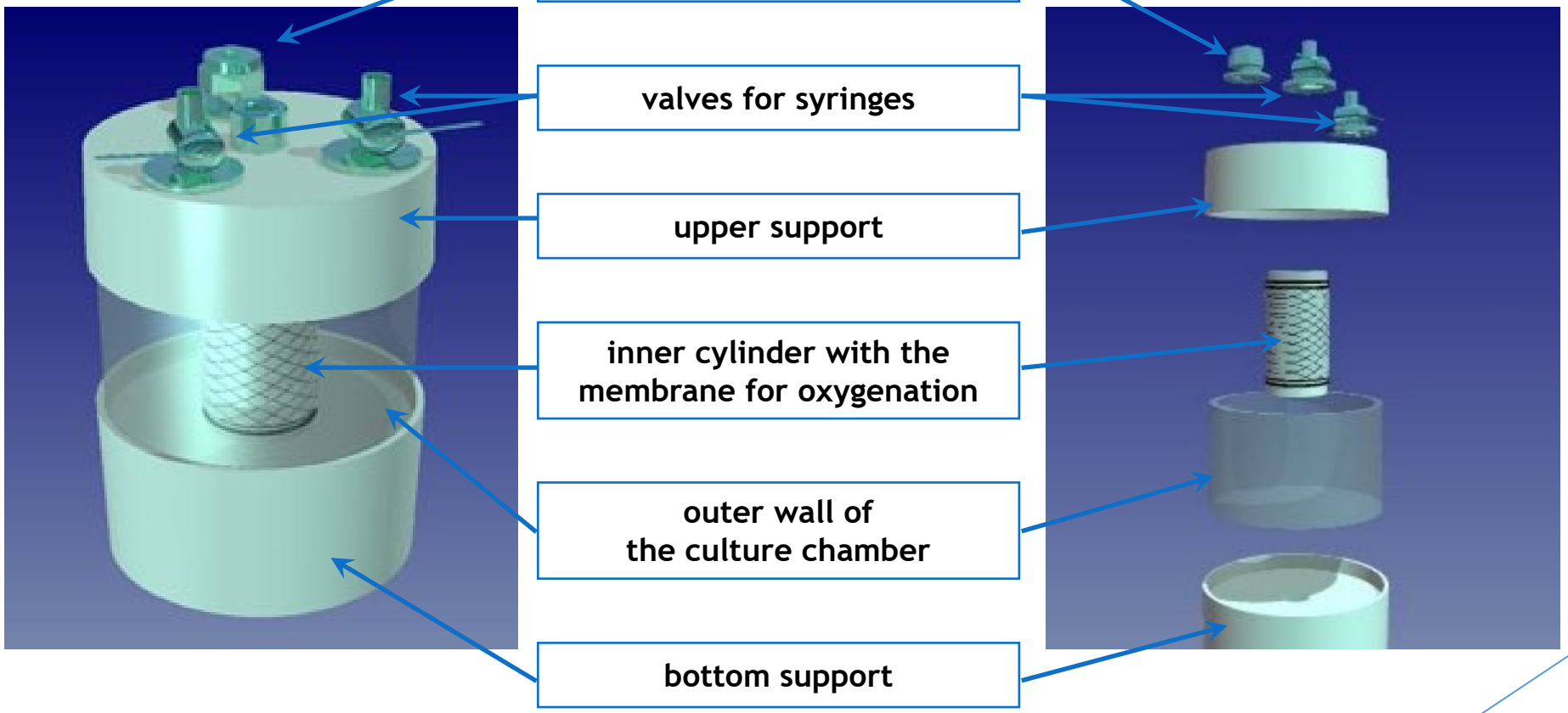
setting the rotating velocity

BASE

motor
shaft
gas pump

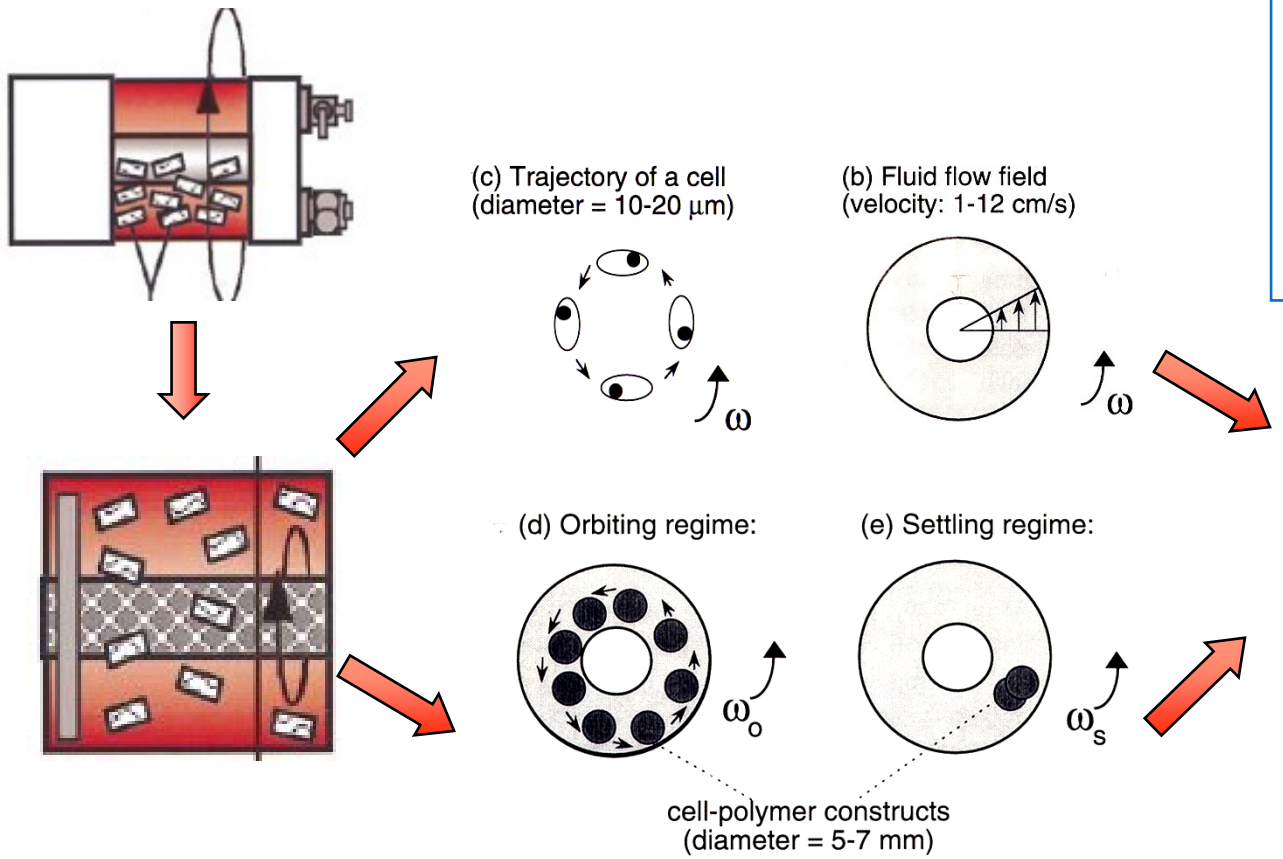


RWV: culture vessel



RWV: working principle

The simulated microgravity is determined by different forces

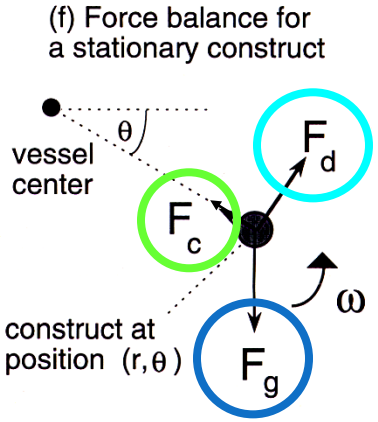


Forces in the culture vessel:

Gravity force (Fg),

Centrifugal force (Fc)

Hydrodynamic resistance force (Fd)



Begley & Kleis *Biotechnology & Bioengineering (2000)*
Hammond & Hammond *Am J Physiol Renal Physiol (2001)*

Rotating Wall Vessel

Rotating Wall Vessel: considerations

The rotating-wall vessel is a **suspension culture vessel** optimized to produce laminar flow and minimize the mechanical stresses on cell aggregates in culture. To minimize mechanical damage and optimize differentiation of cultured cells, suspension culture should be performed in a solid-body rotation Couette-flow, zero-headspace culture vessel such as the rotating-wall vessel.

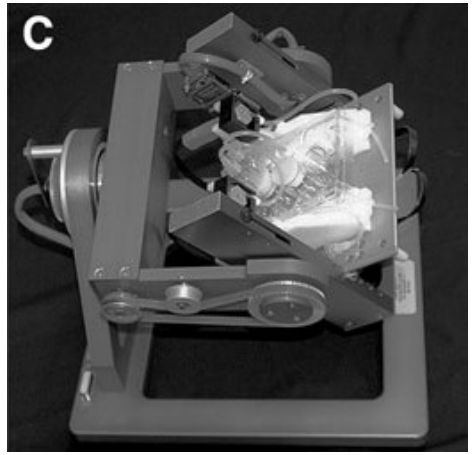
This provides fluid dynamic operating principles characterized by

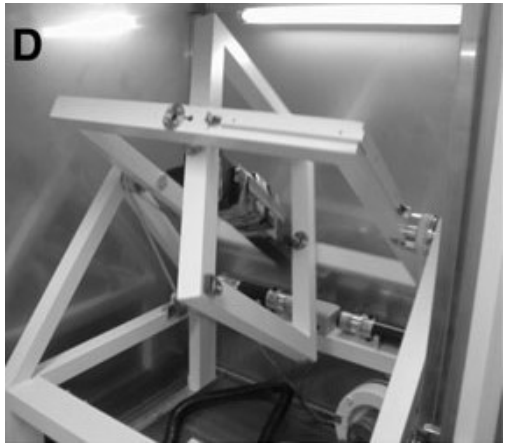
1) solid body rotation about a horizontal axis, characterized by colocalization of cells and aggregates of different sedimentation rates, optimally reduced fluid shear and turbulence, and three-dimensional spatial freedom; and

2) oxygenation by diffusion.

Optimization of suspension culture is achieved by applying three tradeoffs. **A- terminal velocity** should be minimized by choosing microcarrier beads and culture media as close in density as possible; **B- rotation in the rotating-wall vessel** induces both Coriolis and centrifugal forces, directly dependent on terminal velocity and minimized as terminal velocity is minimized, **C- mass transport of nutrients** to a cell in suspension culture depends on both terminal velocity and diffusion of nutrients (limited by the size of cell clusters).

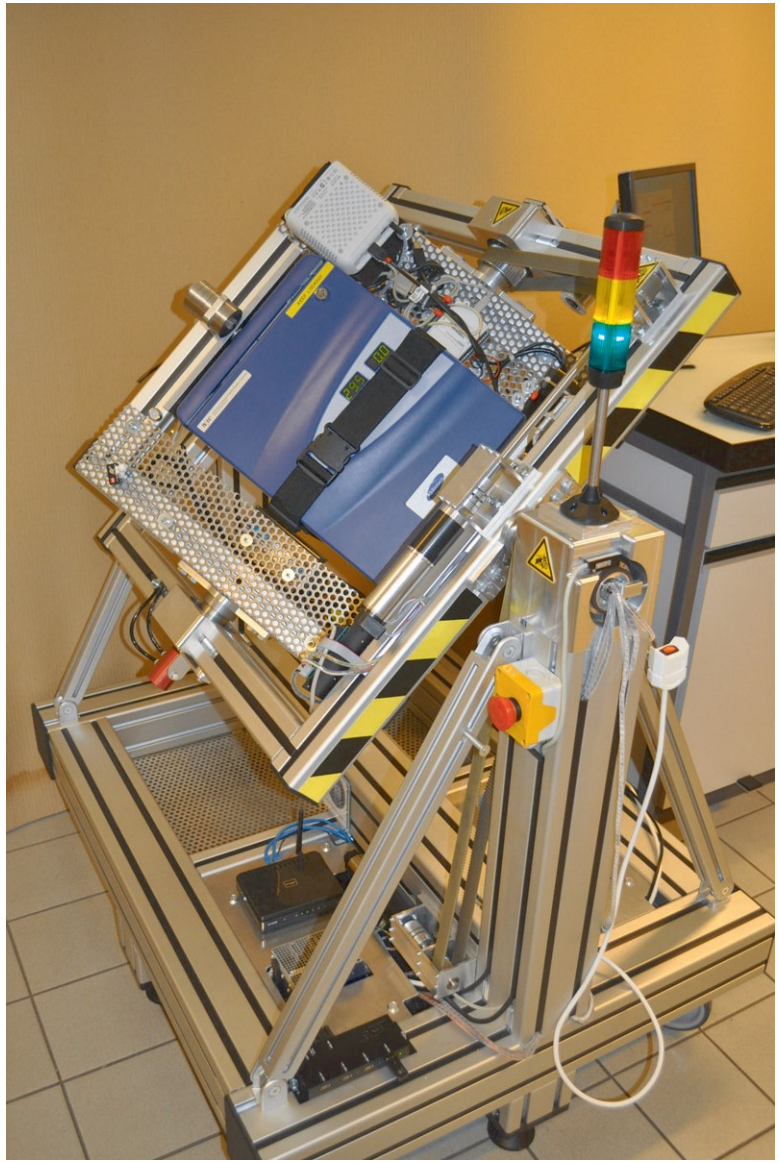
3-D clinostat: Random Positionig Machine





(C) The desktop random-positioning machine (RPM). In this picture the automated fluid managing system COBRA is mounted on the platform together with a standard 12-well tissue culture plate (Image: Dutch Space, Leiden, The Netherlands) (Grimm et al, Tissue Engeenering 2014). (D) the 3-D RPM hosted at DESC/ESA-ESTEC, Noordwijk, the Netherlands (Herranz et al, Astrobiology, 2013).

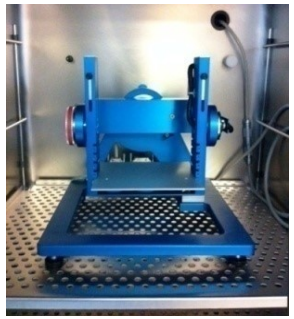
Random Positioning Machine (RPM)



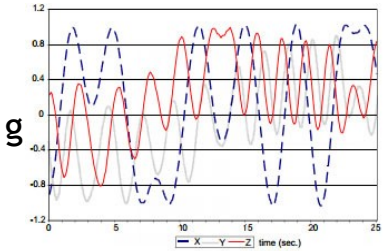
Damm et al Biotech Bioeng 2014

RPM: working principle

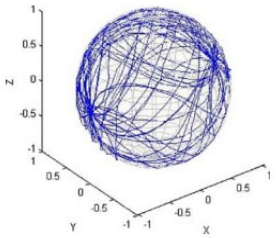
RPM

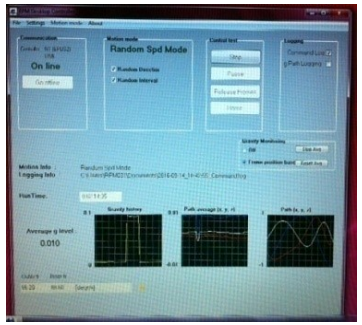


Gravity values measured on the RPM



Motion trajectory in random speed and random direction mode, displayed on an imaginary sphere





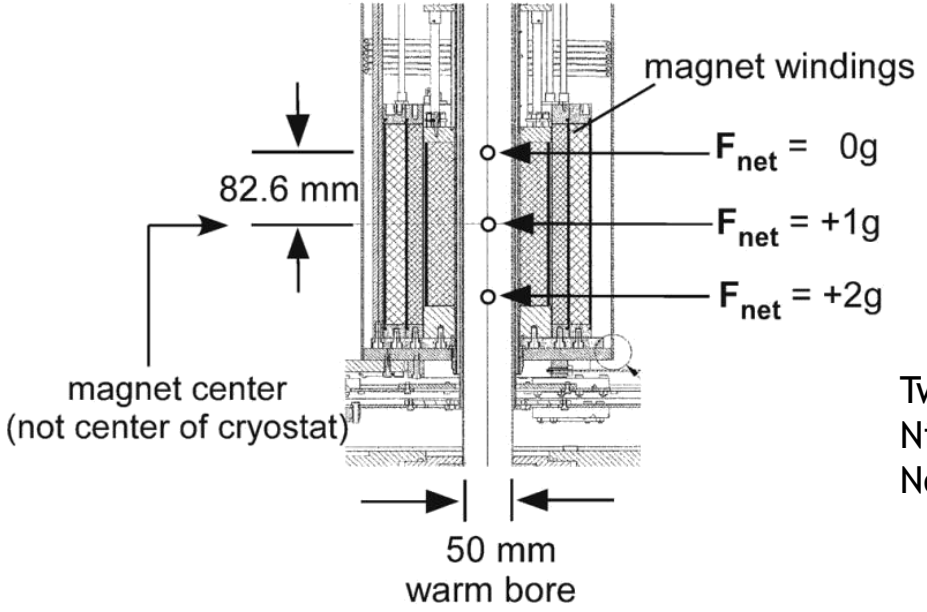
Lab. Functional biotechnology, CAST-University G. d'Annunzio of Chieti-Pescara.
RPM by Dutch Space, Leiden, The Netherlands.
Now: Yuri GmbH, Meckenbeuren, Germany.

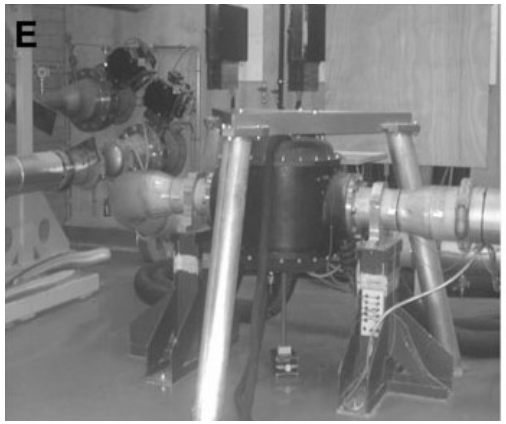
Random Positioning Machine: considerations

Considerations: Cell samples represented by cells in suspension or in adhesion. For obtaining valuable and reliable results from RPM experiments, the appropriate use of the RPM is of utmost importance.

The simulation of microgravity requires that the RPM's **rotation is faster** than the biological process under study, but not so fast that undesired side effects appear. It remains a legitimate question, however, whether the RPM can accurately and reliably simulate microgravity conditions comparable to real microgravity in space. Authors attempt to answer this question by mathematically **analyzing the forces working on the samples** while they are mounted on the operating RPM and by comparing data obtained under real microgravity in space and simulated microgravity on the RPM. In conclusion and after taking the mentioned constraints into consideration, we are convinced that simulated microgravity experiments on the RPM are a valid alternative for conducting examinations on the influence of the force of gravity in a fast and straightforward approach.

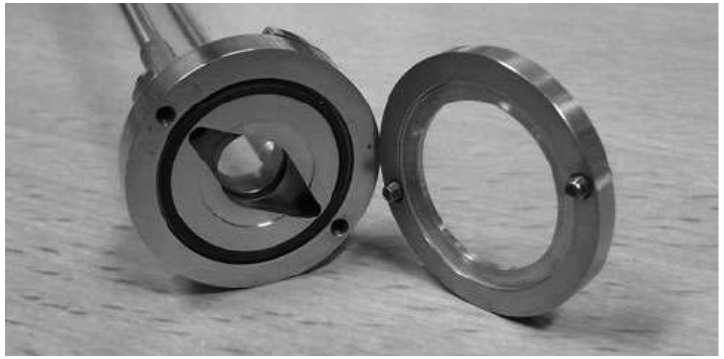
Diamagnetic levitation





Two magnetic levitation facilities hosted at the HFML, Nijmegen, the Netherlands, (E) and the University of Nottingham, UK (F).(Herranz et al, Astrobiology, 2013).

Detailed view of magnet. Samples placed in the warm bore can experience a *magnetic force* from −1 to +1 g, depending on location. The magnetic force vector **(Fm) is collinear to the** gravitational force vector **(Fg), i.e., vertical. The net gravitational force (Fnet) a specimen** experiences ranges from 0 to 2 g, depending on location in the bore (Hammer et al., Microgravity Sci Technol. 21:311, 2009).



Opened culture chamber that was used for experiments in the magnet. The *central circle provides space for a 12 mm* coverslip. After insertion of a coverslip, a glass lid was placed on top of the chamber. The two tubes at the other side of the chamber were used for fluid refreshment, allowing chemical fixation while the experiment was ongoing.

Diamagnetic levitation: considerations

This simulator counteracts the Earth gravity, but it is not possible to distinguish the side-effects of the magnetic field

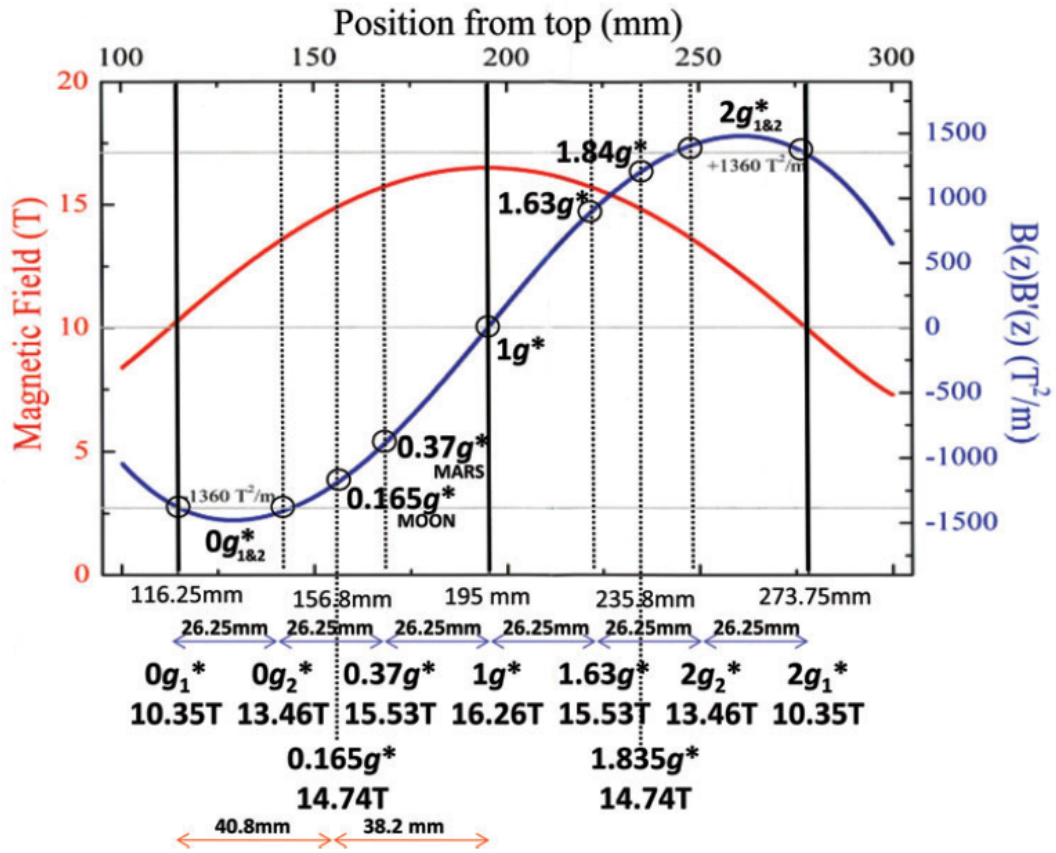
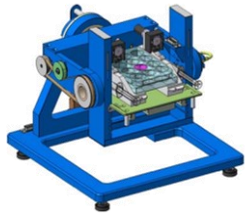


FIG. 2. An example of magnetic levitation experimental positions in relation to the intensity of the magnetic field (curve, left axis) and the net effective force (curve, right axis) along the length of the magnetic bore. Color graphics available online at www.liebertonline.com/ast

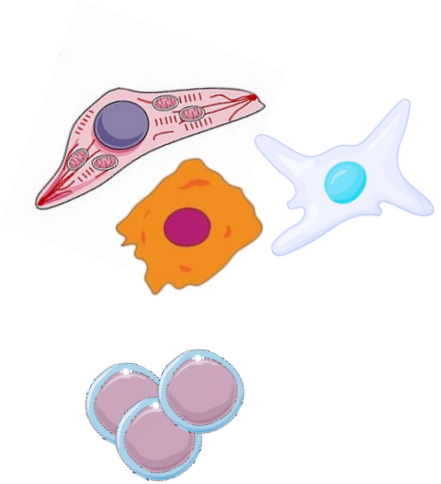
Herranz et al, Astrobiology 13:1, 2013

Our Experimental studies

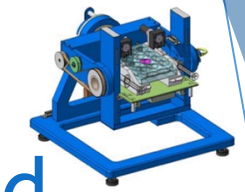
- Simulated microgravity by the Random Positioning Machine (**RPM**)



- Different cell phenotypes: adherent and suspension cells



Comparison of induced simulated microgravity effects between adherent and suspension cells in RPM



CELL MODELS

- Adherent cells
  - TCam-2 male germ cells
  - H9C2 cardiomyocytes
  - MC3T3-E1 osteoblasts
- Suspension cells
  - Jurkat lymphocytes

CONDITIONS

- s-microgravity in RPM ± antioxidants
- Control (Ctr) 1g ± antioxidants

ANALYSIS

- morphological assay
- metabolic assay
- biological assay

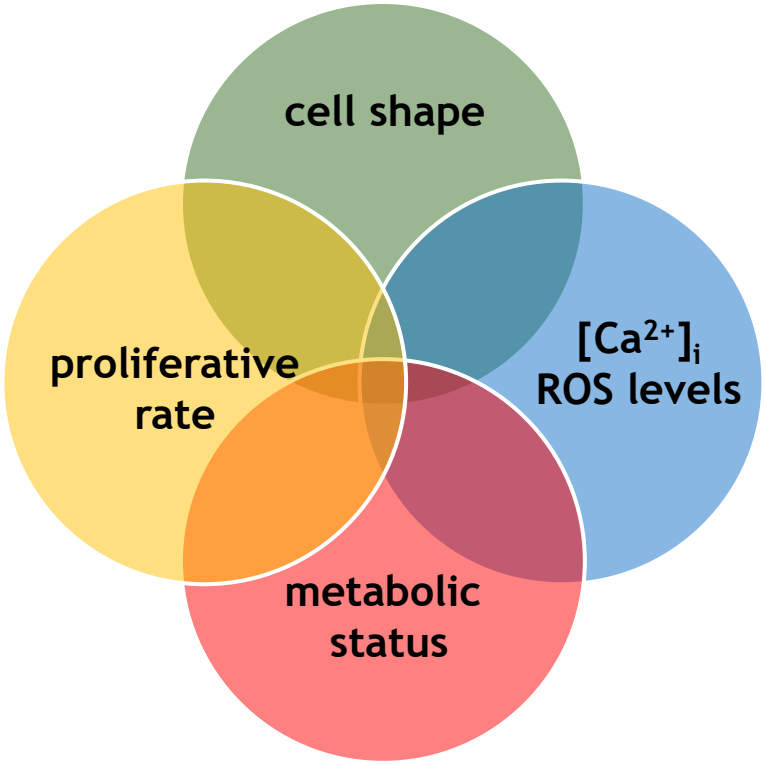
s-microgravity effects

s-microgravity

Affects

antioxidants

For adherent cells

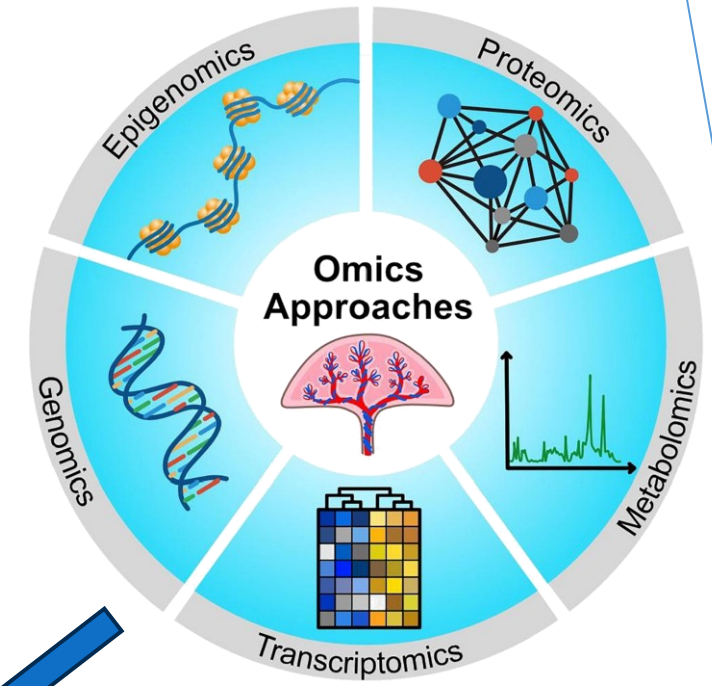


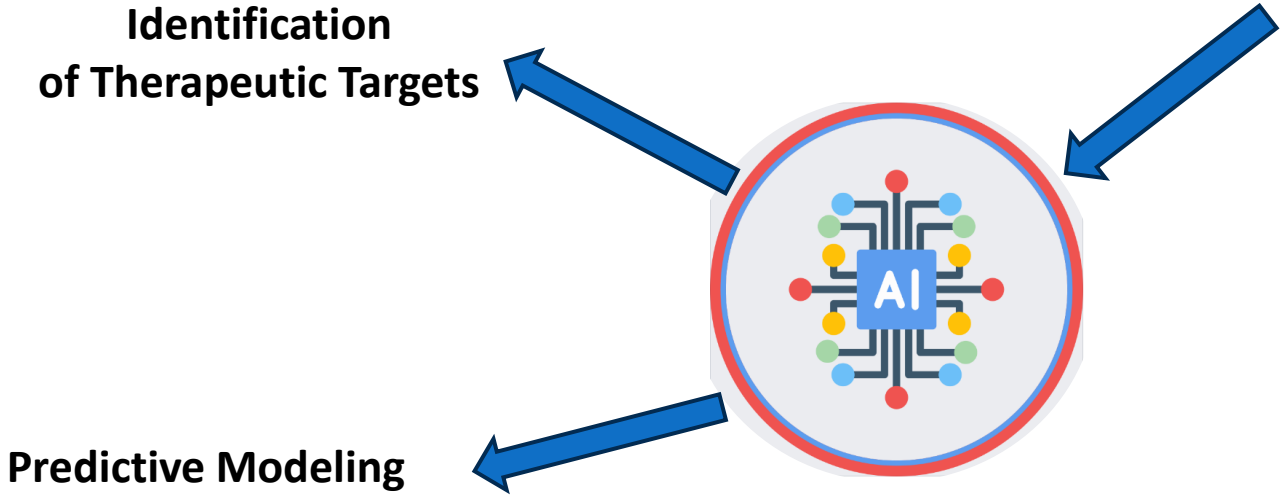
Hypothesis: the different cellular behavior during s-microgravity exposure is dependent on the cell constraints and therefore on the redistribution of external forces

Morabito et al Sci Rep 2017; Morabito et al IJMS 2019 and 2020; Guarnieri et al Oxid Med Cell Longev 2021, Berardini et al Cells 2023

What's the future?

- Multi-omic approaches
- AI analyses





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Dr Nathalie Steimberg

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Thanks for
your attention