Cellular hibernation enables advanced biological research on CubeSat missions

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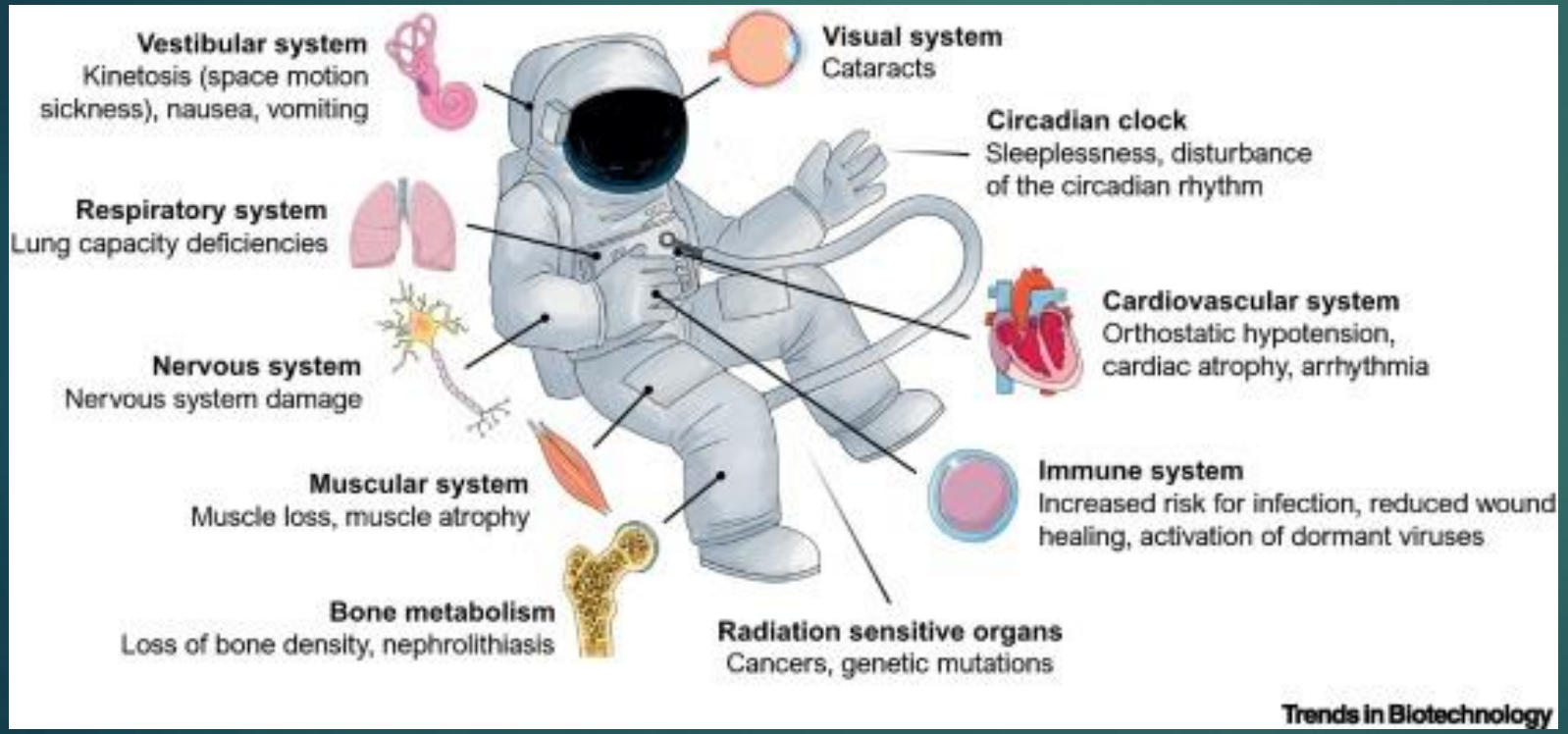
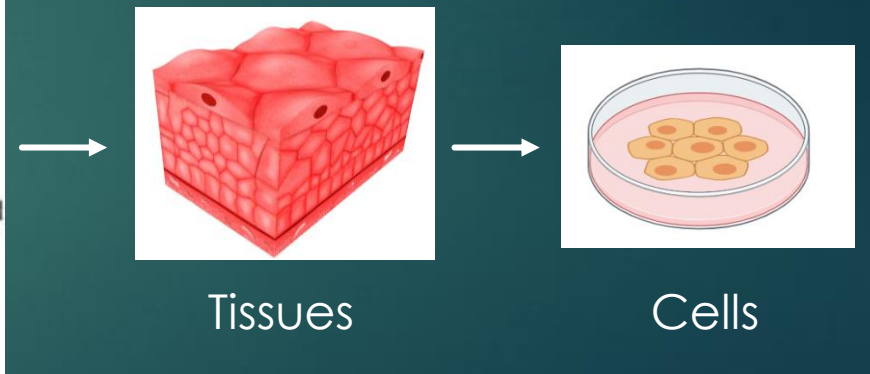
MAYO CLINIC

Why do should we study biology in space?

- Improve understanding of how spaceflight affects living systems
- how long people can survive the extreme conditions
- how fast they can adapt to the Earth's environment after returning





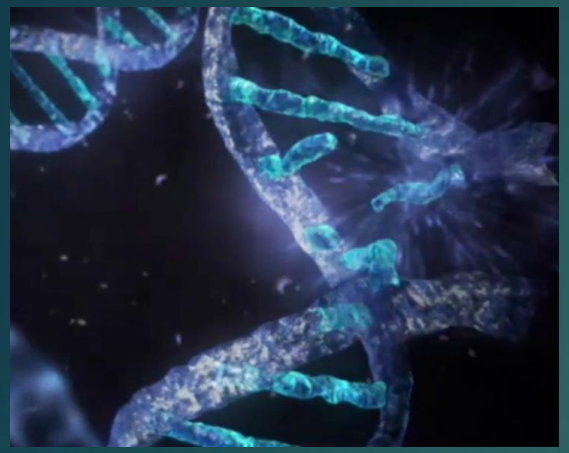
Tissues

Cells

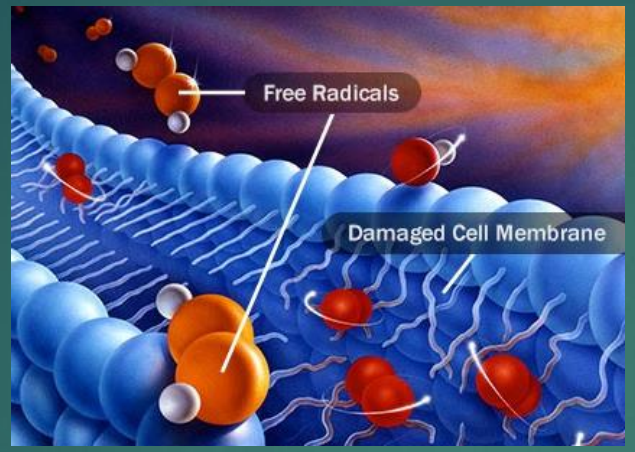
https://www.cell.com/trends/biotechnology/fulltext/S0167-7799%2821%2900195-5
https://www.popularmechanics.com/space/moon-mars/a21330/nasa-wants-martian-resources-for-martian-colony/

What do we study when talking about human biology?

DNA damage



Oxidative stress



Mitochondrial dysfunction

Shifts in the microbiome

Gene regulation

Protein expression

How organisms repair cellular damage and protect themselves from infection and disease?

https://arstechnica.com/science/2022/11/the-mysteries-of-the-astronaut-biome/?comments=1&comments-page=1
https://www.samuelmaddockhealth.com/functional-medicine/how-to-support-your-mitochondria-with-functional-medicine

https://www.nasa.gov/exploration/humanresearch/multimedia/images/hrpg_img_09.html
https://www.northraleighperio.com/blog-news/how-to-fight-oxidative-stress/

Current state | Challenges

Space:

- Limited knowledge of impact of deep space on humans
- Limited ability to adequately maintain human cells autonomously
    - Cells require temperature control, oxygen, and nutrients

Earth:

- established cell culturing methods and protocols

Goal:

- provide the basis for a controlled environment that increases science return to study disease development and risk mitigation

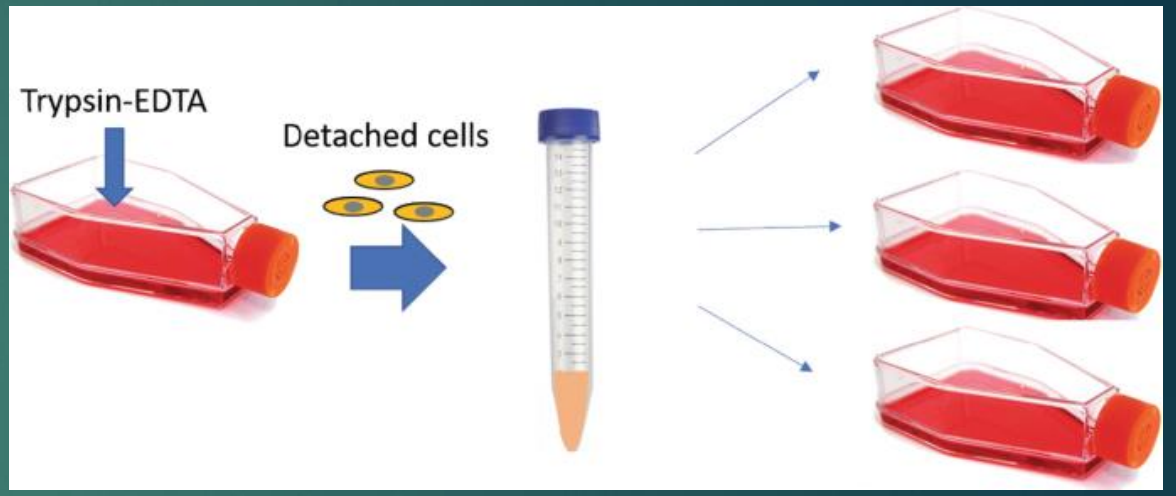
What are key elements to studying human cells?

- Cells that continue to grow

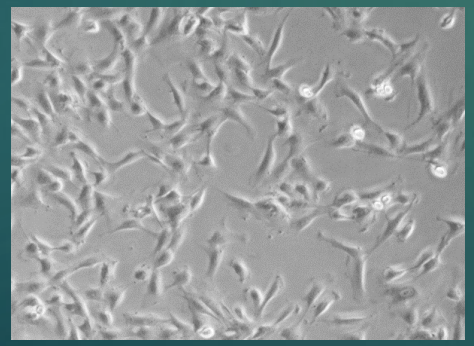
Day 0 - Plate

Day 2-3 - Feed

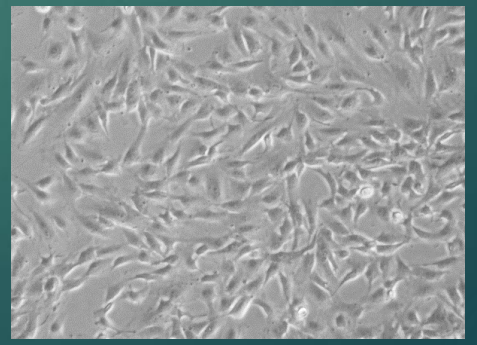
Day 4-5 - Subculture (split) -(80%)



Attached cells

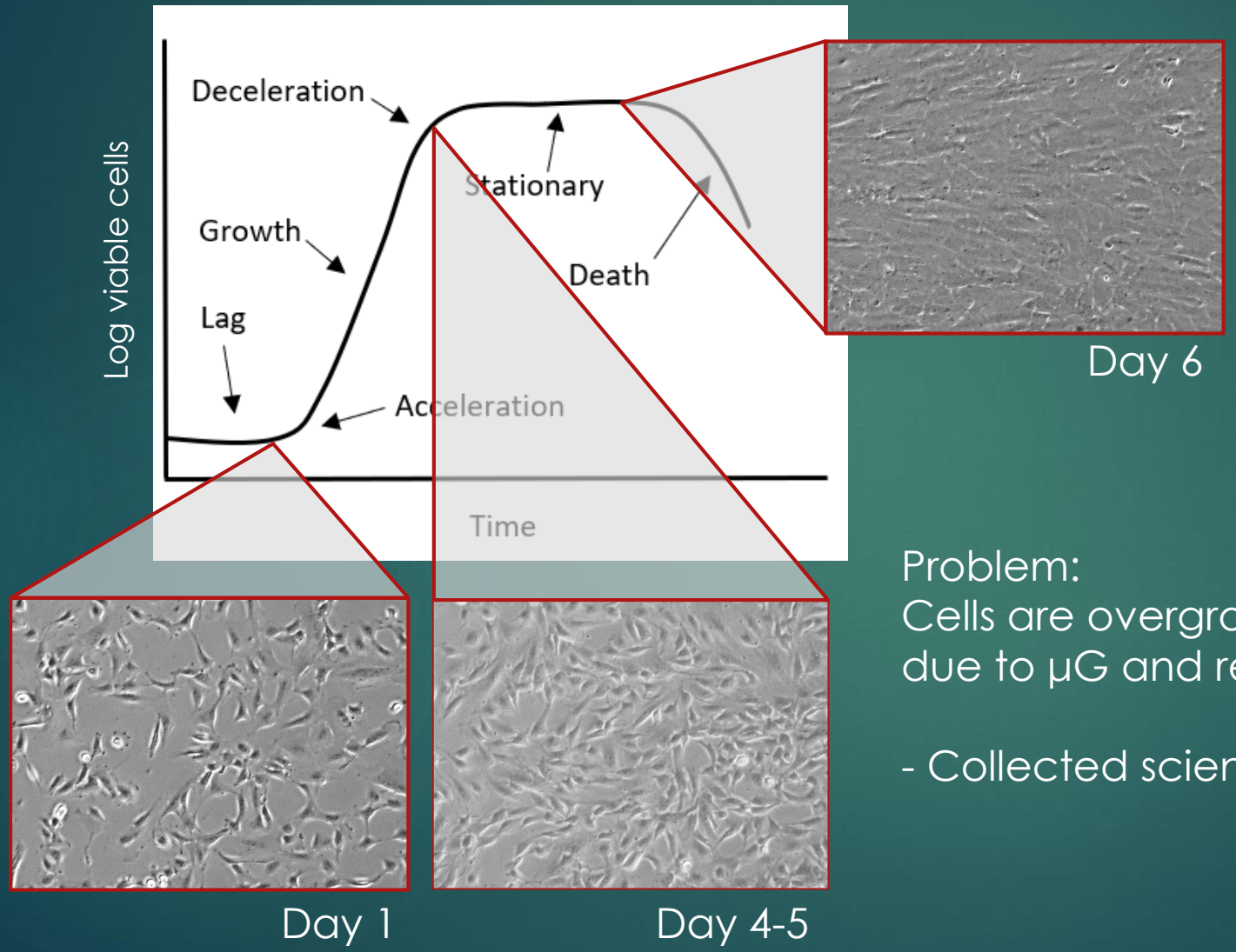


Ready to split



https://www.genengnews.com/magazine-issues/september-15-2015-vol-35-no-16/feng-shui-basics-for-3d-cell-culture/
https://link.springer.com/chapter/10.1007/978-3-030-83696-2_3

What is the challenge when studying human cells in space?



Problem:
Cells are overgrown and cannot be split due to µG and required astronaut time

- Collected science might be impaired

What is the challenge when studying human cells in space?

Growth and inadequate opportunity to split

>5d ISS

>10d Moon

?d

Example for an ISS mission:
T-3 cells are plated
T-2 handover and integration
T+2 arrival on ISS and change of nutrients (**total 5d**)
T+7-10 experimental data gathering

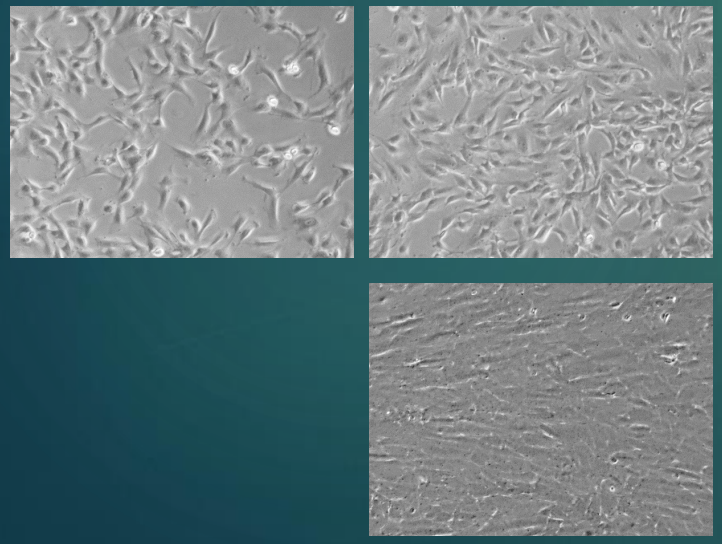
https://www.nature.com/articles/s41526-020-0106-z/figures/1
https://toppng.com/free-image/nasa-iss-2011-PNG-free-PNG-Images_67434

What is the challenge when studying human cells in space?

Growth and inadequate opportunity to split

>5d >10d ?d

Day1 Day5



During transport:
Feeding
Overgrown on arrival

Current approach:

Challenge:
Suboptimal cell density for maintenance of cell-cell contacts

https://www.nature.com/articles/s41526-020-0106-z/figures/1
https://toppng.com/free-image/nasa-iss-2011-PNG-free-PNG-Images_67434

Solution

Create the state of low metabolic activity or hibernation to reduce:
- nutrient need
- cell growth
- toxin production

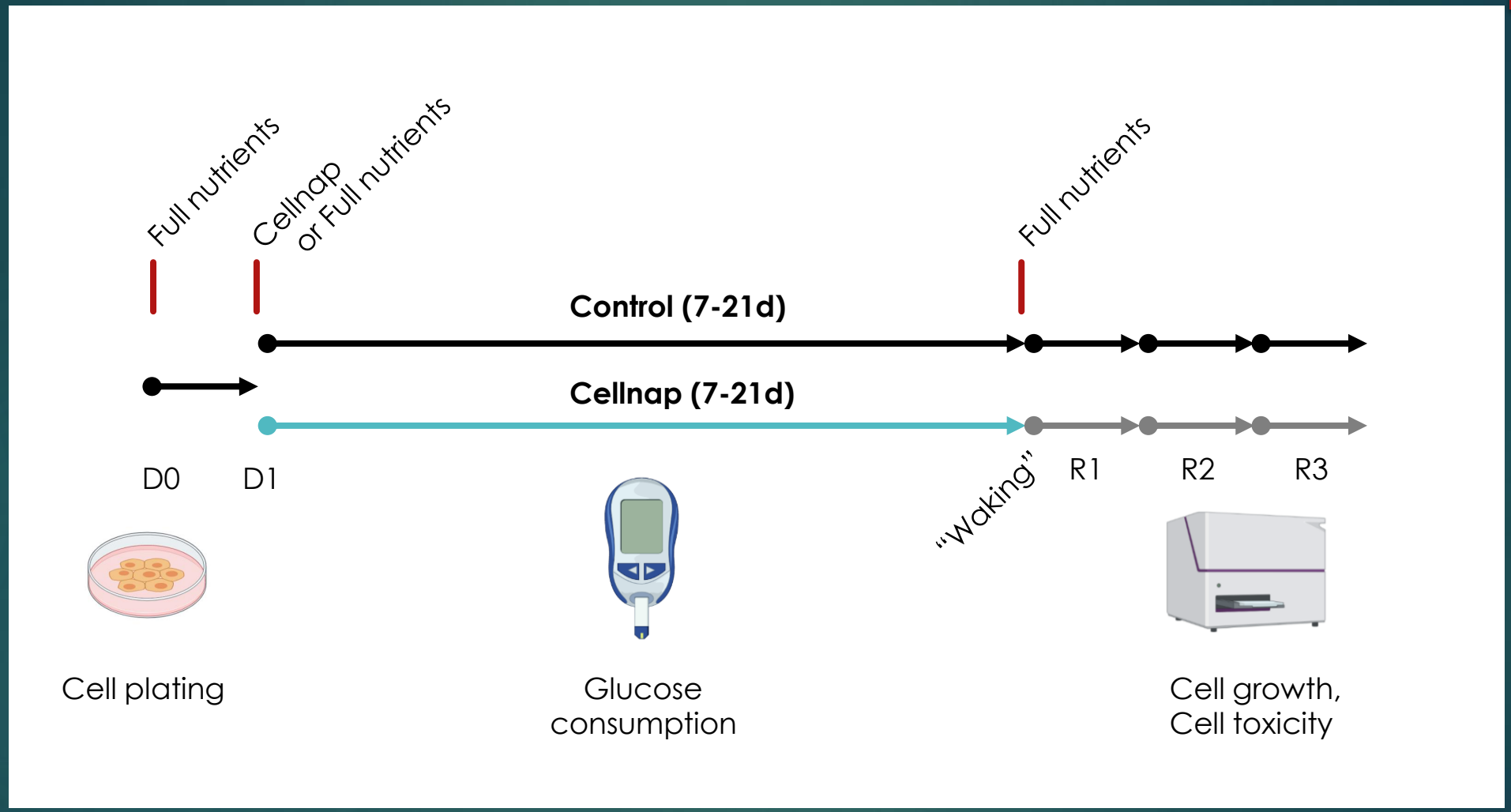
Special considerations:
- Possibility of keeping single constant temperature
- No fluid exchange needed
- On-demand “waking” of the cells for in orbit experiments

Identification of a hibernation solution:

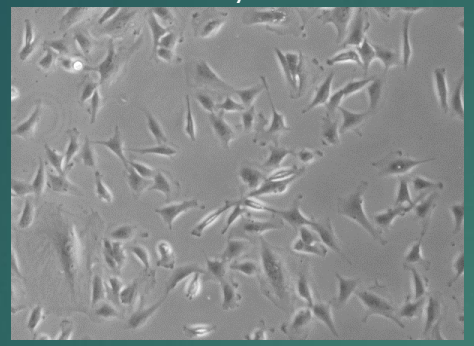
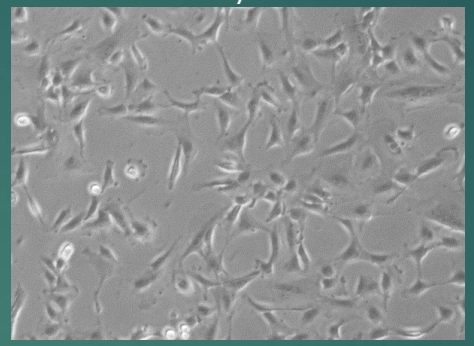
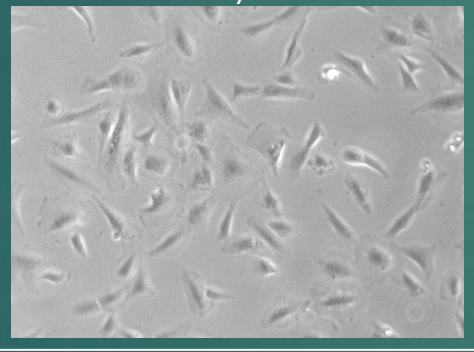
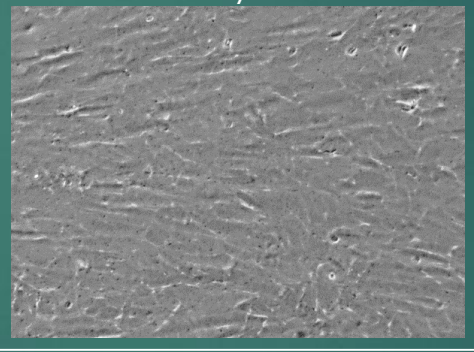
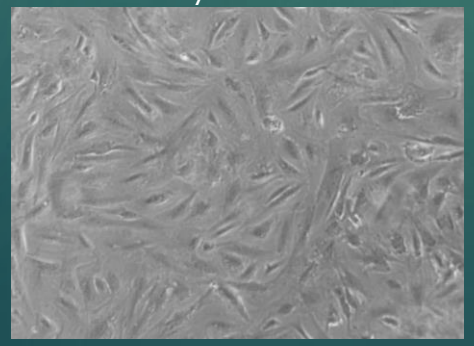
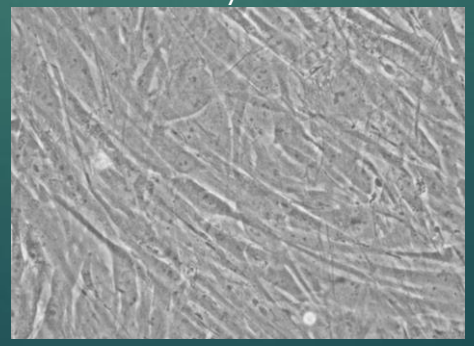
CELLNAP

https://blog.bioticsresearch.com/7-ways-to-promote-stem-cell-proliferation

Design


Full nutrients
Cellnap
or Full nutrients
Full nutrients
Control (7-21d)
Cellnap (7-21d)
D0
D1
"Waking"
R1
R2
R3
Cell plating
Glucose
consumption
Cell growth,
Cell toxicity

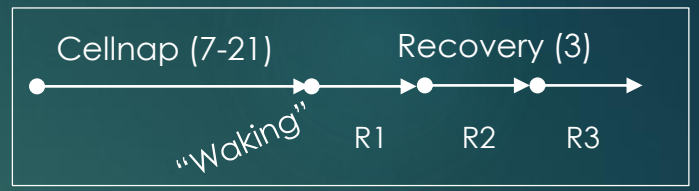
Evaluation – cell morphology after 21 days in Cellnap

Cellnap	Control
Day: 1	Day: 1
	
Day: 7	Day: 7
	
Day: 22 R1	Day: 22
	

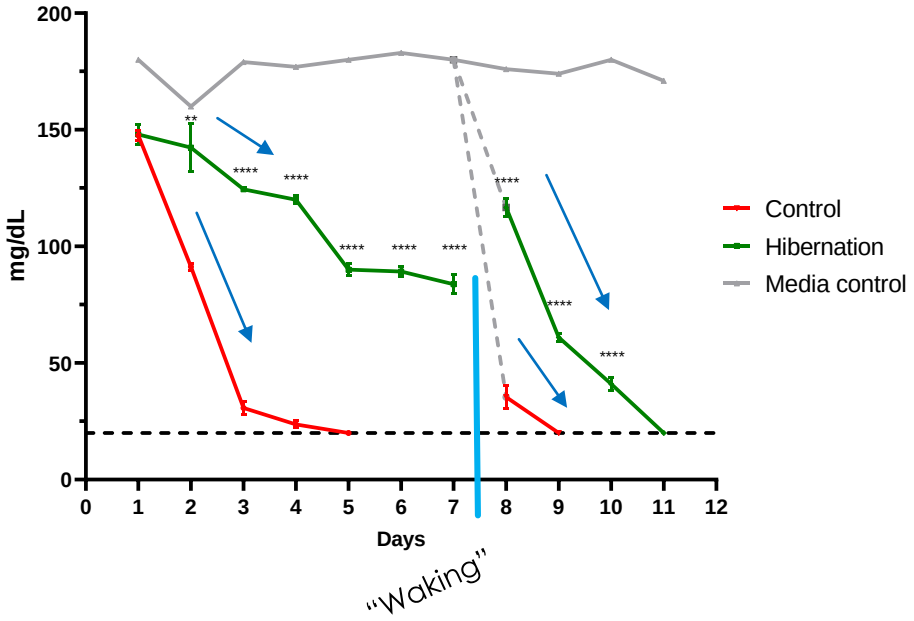
Human Hepatocytes

Evaluation

Human Hepatocytes



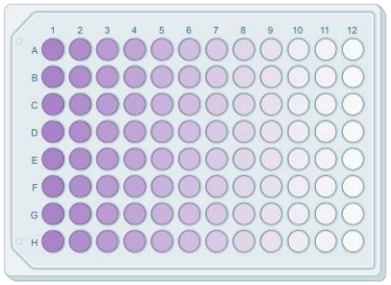
Glucose Consumption



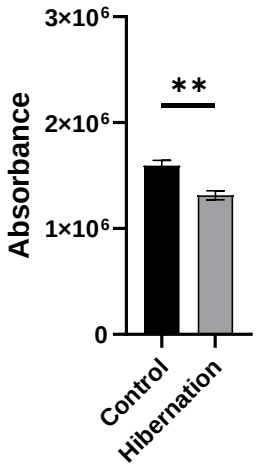
Rate change

	Day 1-3 (mg/dL)	Day 8-10 (mg/dL)
Control	36.30	35.33
Hibernation	7.70	38.98

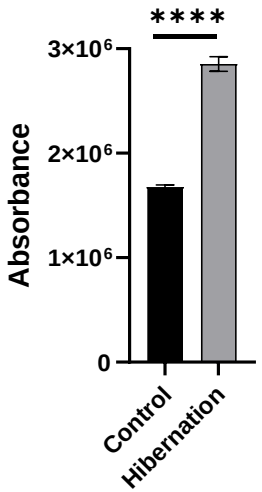
Cell growth



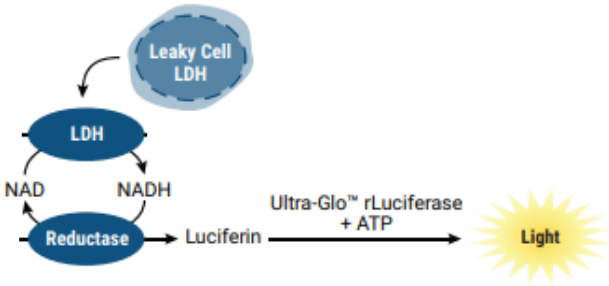
Hibernation D7

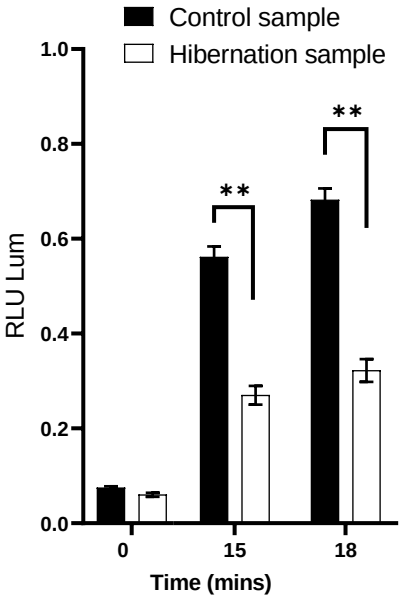


Recovery Day: 3



Cell membrane damage



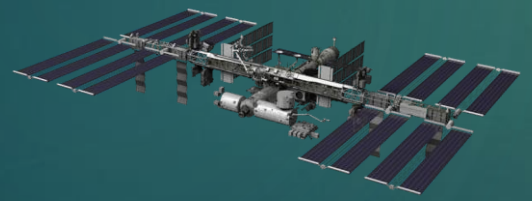


https://www.aatbio.com/resources/application-notes/mtt-assay \https://www.promega.com/products/cell-health-assays/cell-viability-and-cytotoxicity-assays/ldh-glo-cytotoxicity-assay/?catNum=J2380#protocols

Putting things into context – addressing the Challenges

- Tested solution on over 5 different cells types
- Can hibernate cells successfully for >10d
- Recover fully after 24-48h and ready for experiment

Practical approach for an ISS mission CubeSat deployment:
T-3 cells are plated
T-2 cells are place into Cellnap
- handover and integration
T+2 arrival on ISS and "waking"
T+7-10 experimental data gathering



Practical approach for lunar and CubeSat missions:
T-3 cells are plated
T-2 cells are place into Cellnap -
handover and integration
T+10 arrival on the lunar surface and "waking"
T+17-20 experimental data gathering

Possibility for on-demand staggered experiment starts and repeat experiments

http://clipart-library.com/clipart/1670097.htm

Going further…

- Continue to optimize and test Cellnap (potentially modify for longer duration)
- More extensive cell analysis including mitochondrial health, gene analysis, proteomics
- Terrestrial interest

https://www.istockphoto.com/photos/reach-for-the-stars

Thank you!

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https://www.nasa.gov/sites/default/files/thumbnails/image/mars_base1.jpg