COLLEGES OF NANOSCALE
SCIENCE + ENGINEERING
SUNY POLYTECHNIC INSTITUTE

COMSOL CONFERENCE
2018 BOSTON

Analyzing Fluid Shear Stress in the RCCS: Applications for 3D Cell Culture in Simulated Microgravity

Presented by Timothy Masiello, MS

SUNY Polytechnic Institute,

Colleges of Nanoscale Science and Engineering

The Collaborative Team

SUNY Polytechnic Institute

Role: Core experimental design, preliminary experiments

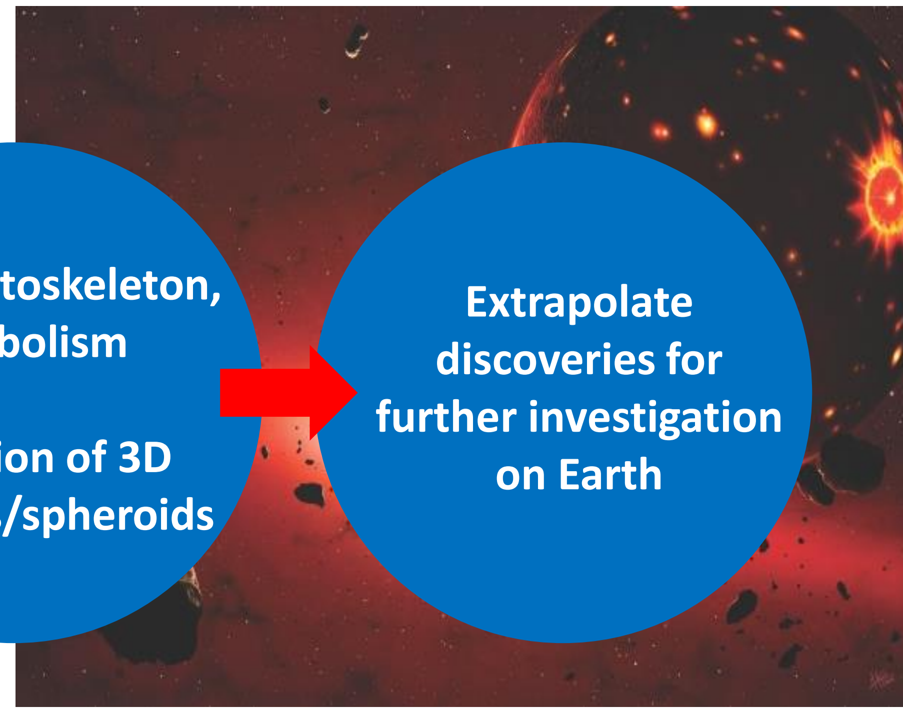
SpacePharma, Inc.

Role: Building/testing end-to-end system for low-earth orbit studies

Microgravity: Why Should We Care?

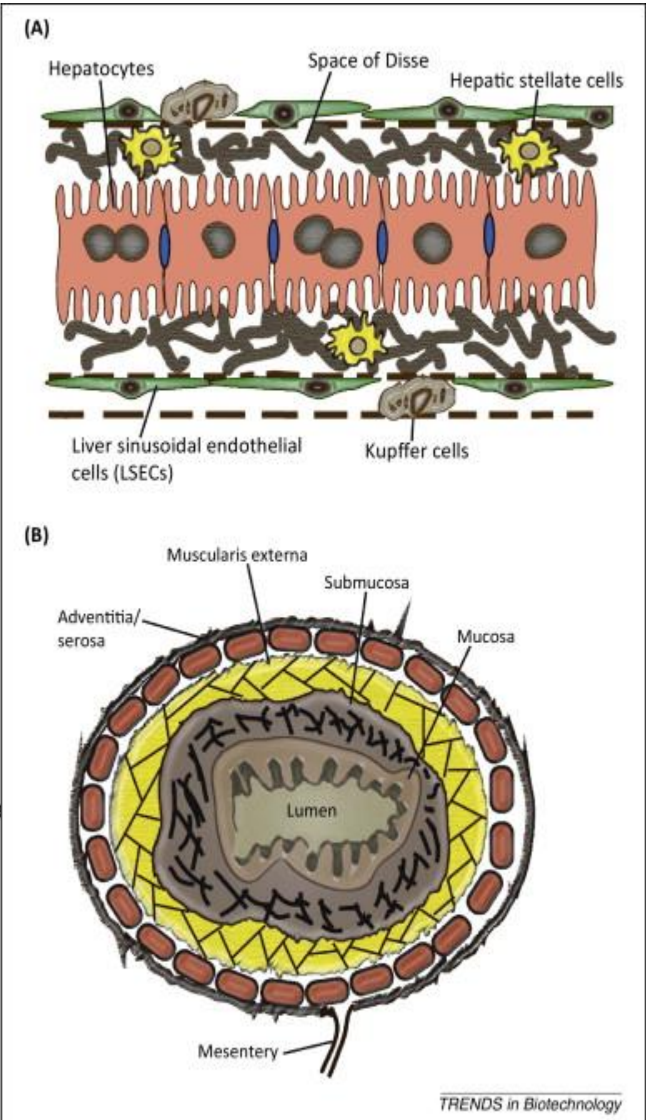
- Definition: when the acceleration of gravity ranges from 10^{-4} to 10^{-6} g
- Organisms on Earth are subjected to 1 g; microgravity is experienced in space and on various celestial bodies.
- Can be simulated on Earth in numerous ways (usually can get down to 10^{-3} to 10^{-4} g at best).
- Applications include:
  - Colloid ... ions
  - Pr...
  - F...
  - T...
  - Ce... ging

Cells in microgravity (real/simulated) → Altered cytoskeleton, metabolism; Formation of 3D organoids/spheroids → Extrapolate discoveries for further investigation on Earth

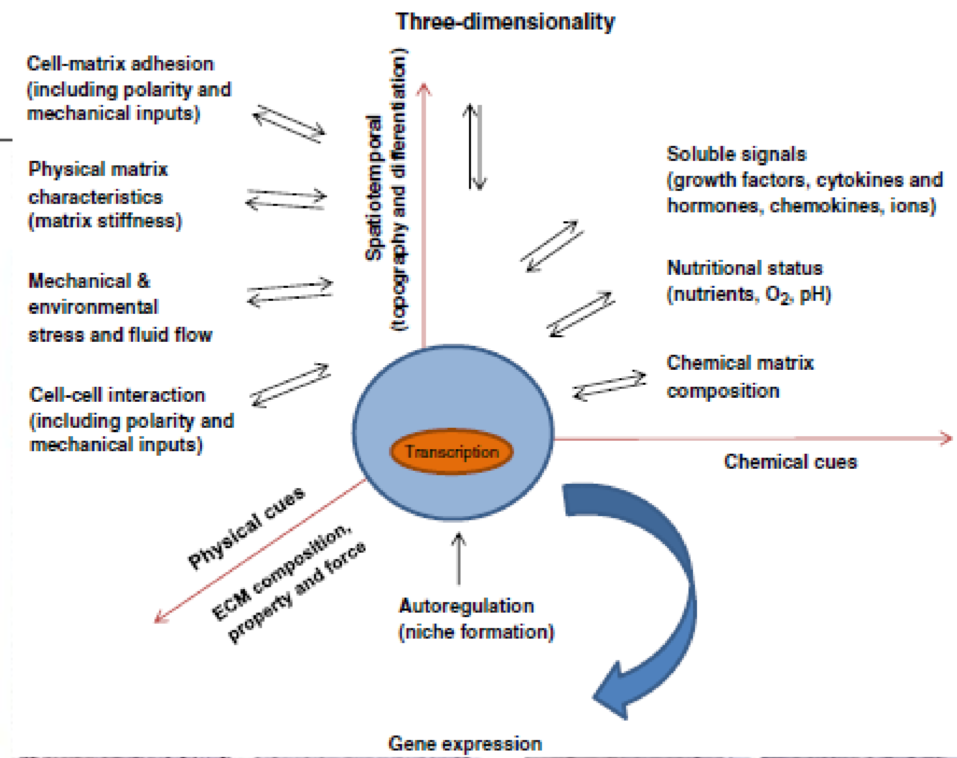


www.spaceanswers.com

Microgravity: The Isolation of Gravity as an Experimental Variable



Vu et al., 2014



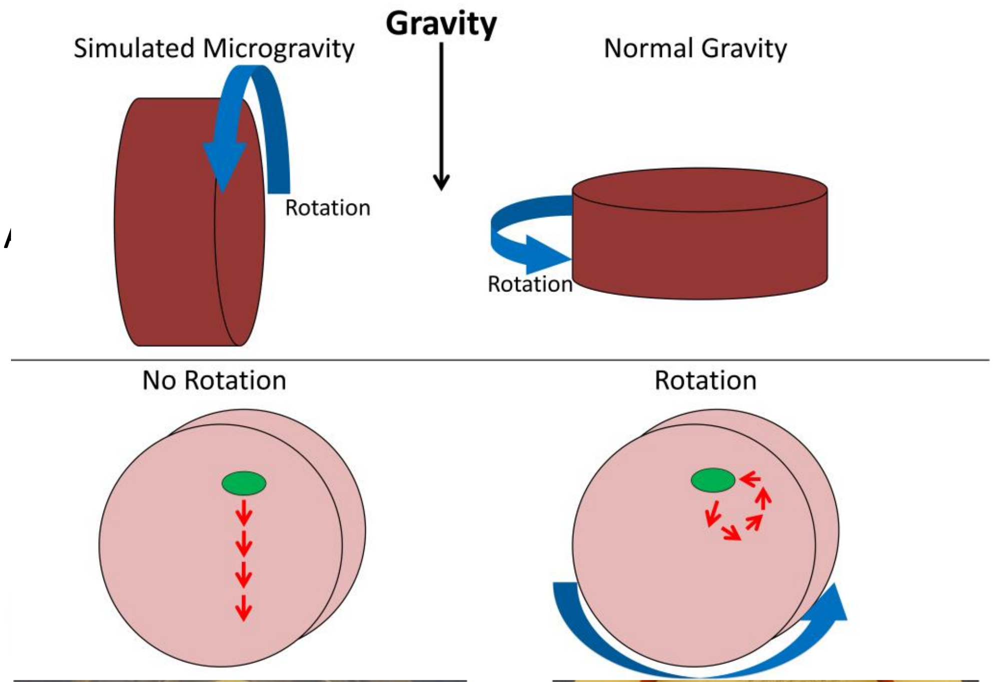
Li and Cui, 2014

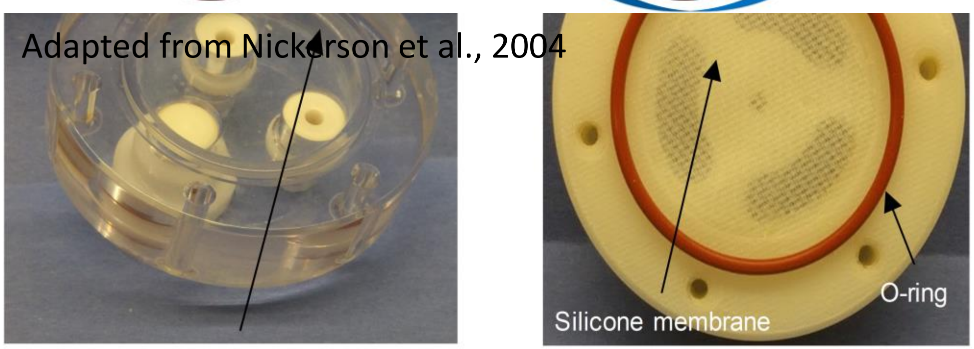
Cerwinka et al., 2012

Adapted from Ebara, 2015

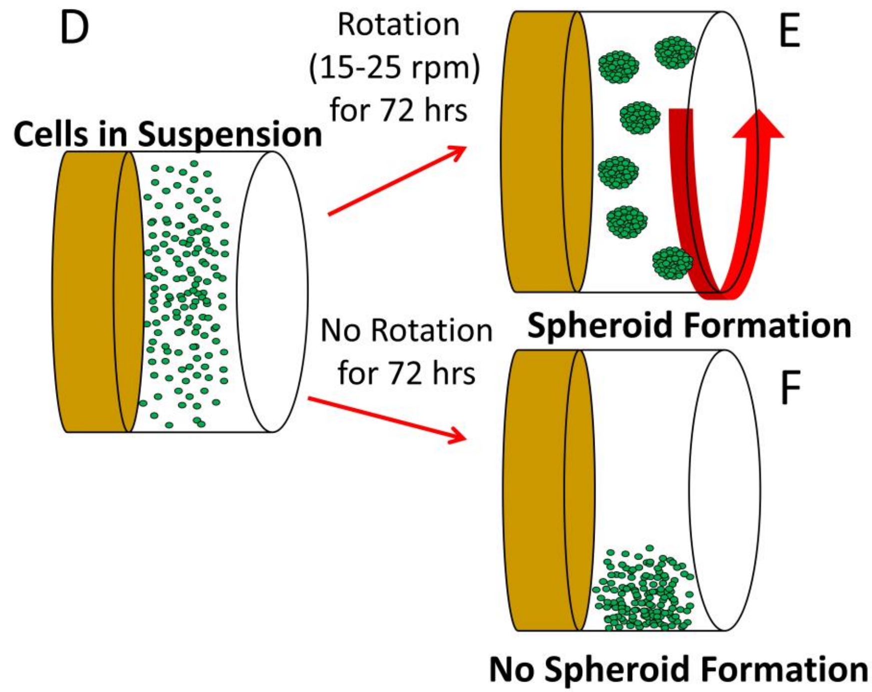
***Spheroids: three-dimensional cell aggregates**
Better representation of *in vivo* environment *in vitro
***Useful tools for studying cancer, organogenesis, etc.**

The Rotary Cell Culture System (RCCS) Simulates Microgravity on Earth





Adapted from Nickerson et al., 2004



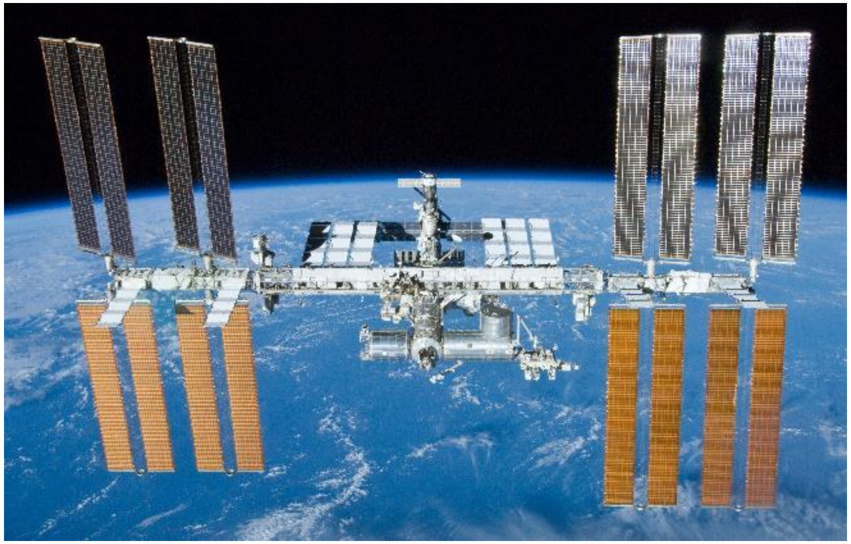
***Balance of centrifugal and gravitational forces**
***Coriolis forces act on spheroids**
***Spheroids experience low shear stress as they rotate (why this can be considered simulated microgravity)**
***This will vary based on spheroid size**

Role of COMSOL Multiphysics ®

-Space-based experiments are the primary goal

-These experiments are expensive, difficult to schedule and challenging to design

-Need: consistent, reliable Earth-based simulation experiments



Wikipedia.org

Experimental Challenge: If low shear stress is crucial to microgravity simulation, how can we maintain consistent, low shear stress in the RCCS, namely across spheroids of various sizes?

Proposed Solution: Model fluid and particle dynamics using the COMSOL ® Multiphysics 5.3 Computational Fluid Dynamics (CFD) and Particle Tracing modules to show how adjusting rotational speed and media viscosity can improve experimental consistency.

Computational Fluid Dynamics (CFD)

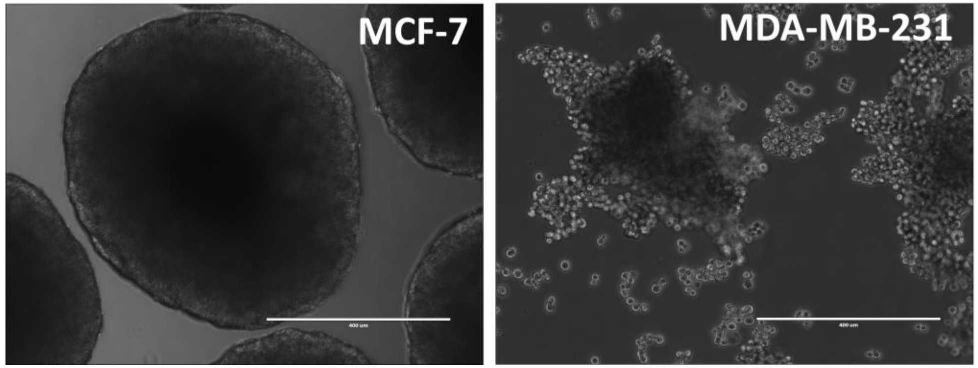
Single-phase flow (spf) studies using base parameters for cell culture media (DMEM) under laminar conditions were used to measure the shear stress distribution across the vessels across different rotation speeds and viscosities.

Particle Tracing

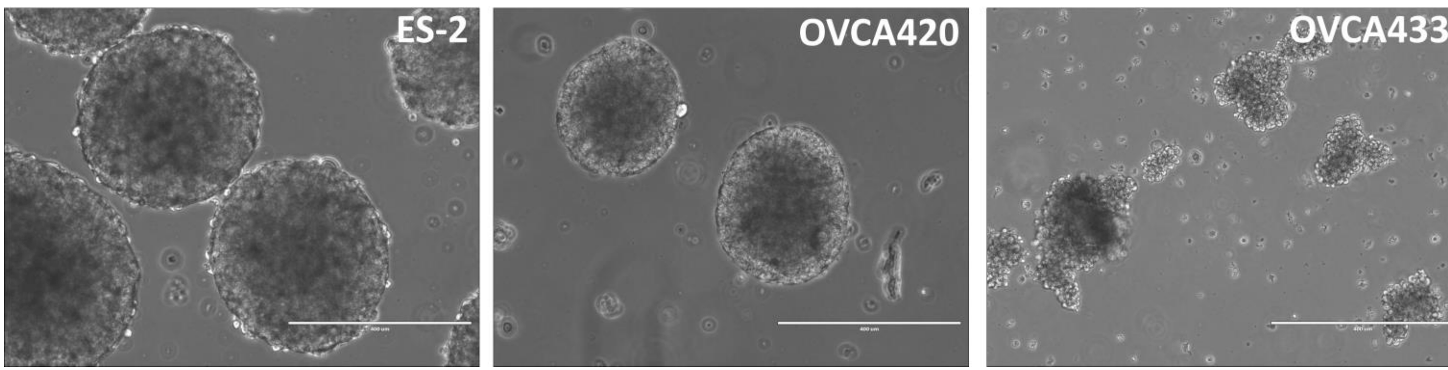
Particle sizes were chosen based off previous studies/literature. This was used to show spheroid positioning and experienced shear stress within vessel.

Spheroids Generated With the RCCS

Breast Cancer



Ovarian Cancer

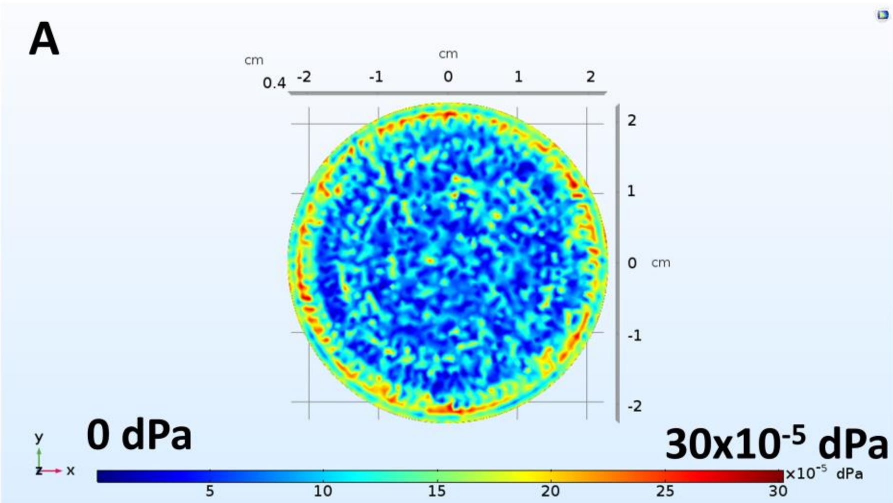


Scale Bar: 400 µm

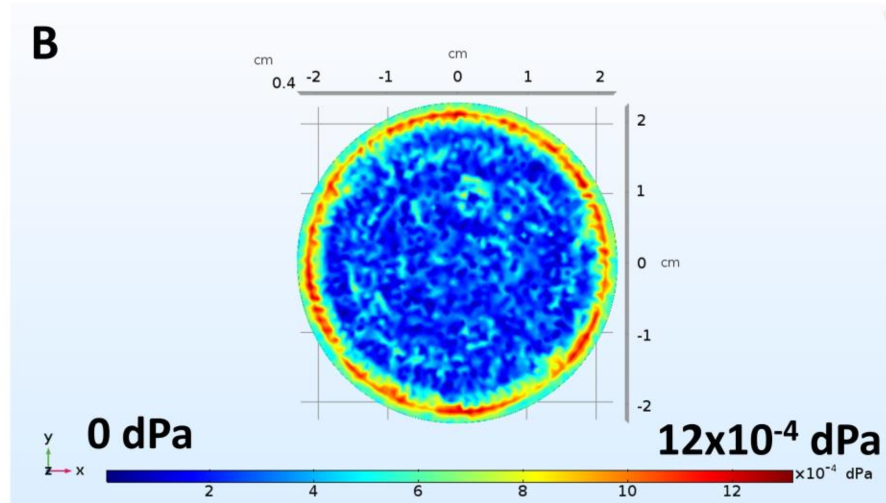
***Cells form a variety of sizes/morphologies of spheroids**
***Can we modulate the shear stress to ensure consistency across these sizes?**

Fluid Shear Stress Distribution Within A HARV (Baseline)

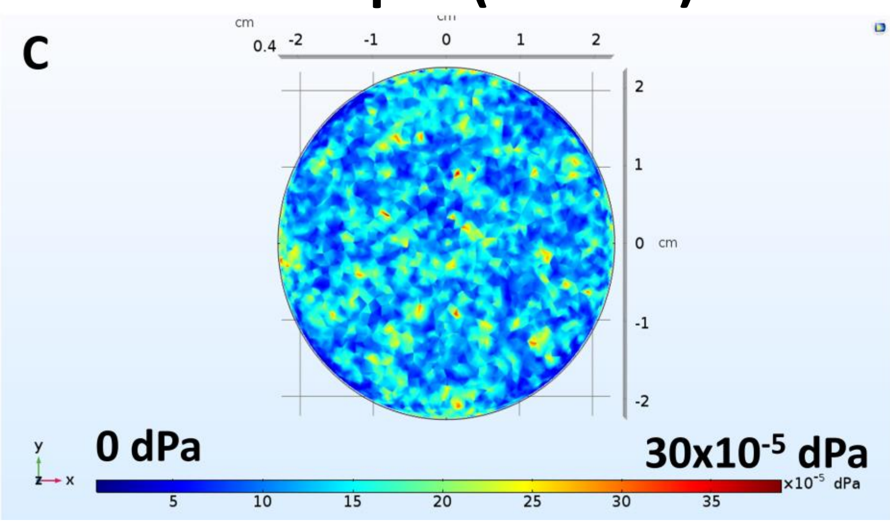
15 rpm (Bottom)



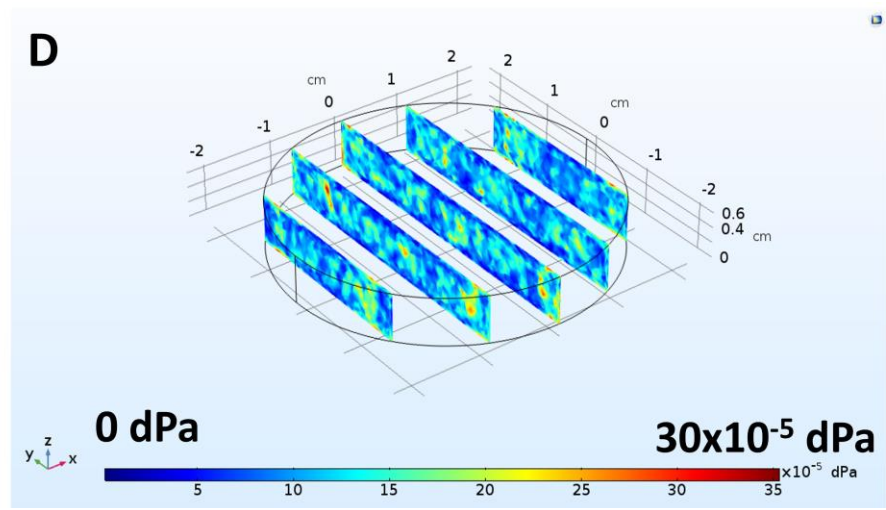
25 rpm (Bottom)



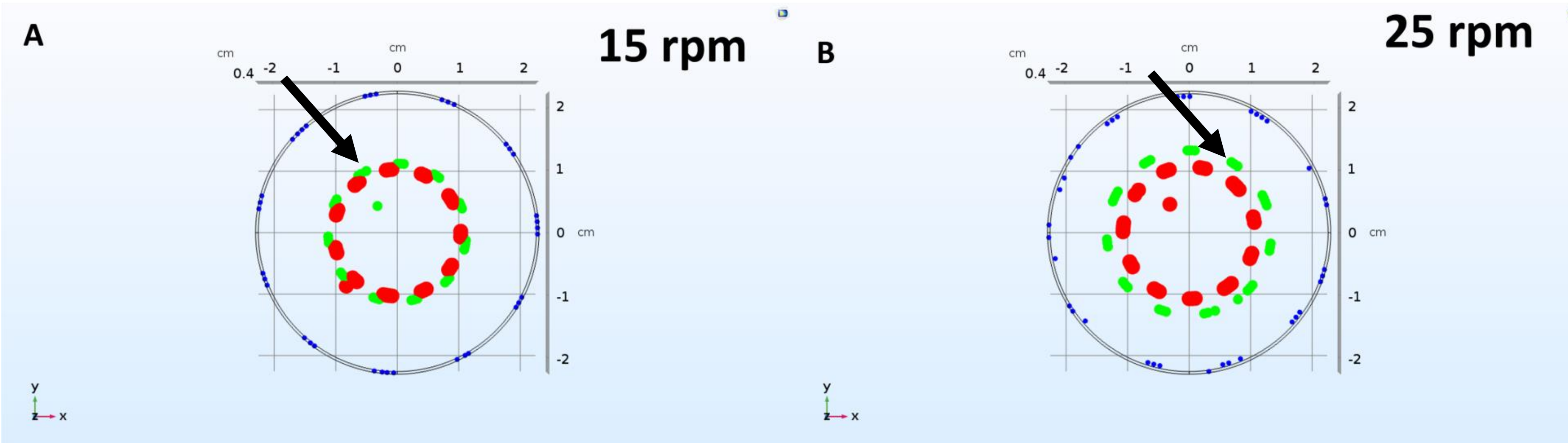
15 rpm (Middle)



15 rpm Cross-Sectional



Particle Tracing For Multiple Spheroids Within A HARV (Baseline)



Radius

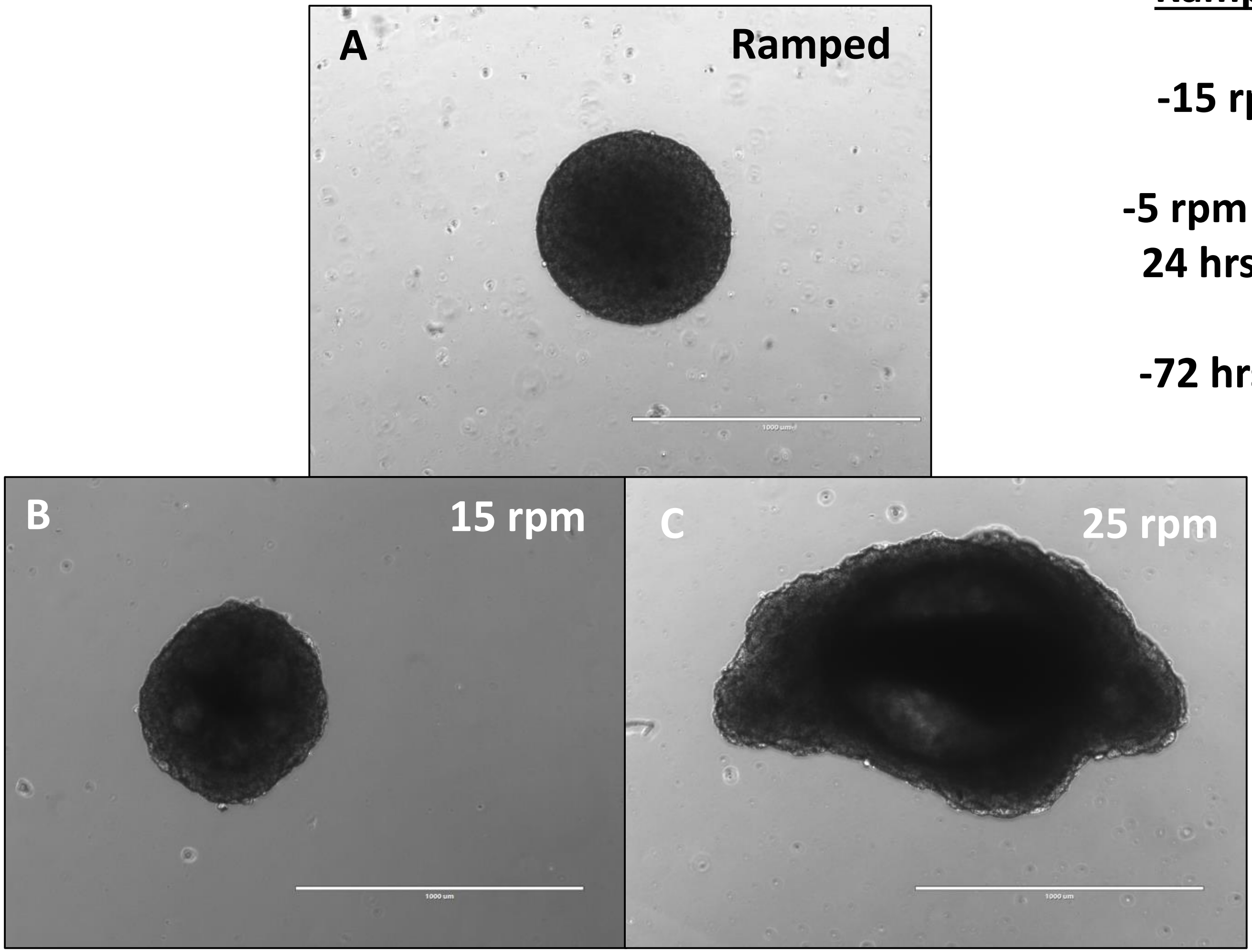
50 µm

100 µm

200 µm

***At certain sizes, spheroids deviate from the HARV's center with increasing speed, increasing shear stress**
***We want to maintain/restore the lower shear stress seen at 15 rpm when the speed is 25 rpm**

Higher Shear Impacts Spheroid Morphology + Behavior



Scale Bar: 1000 µm

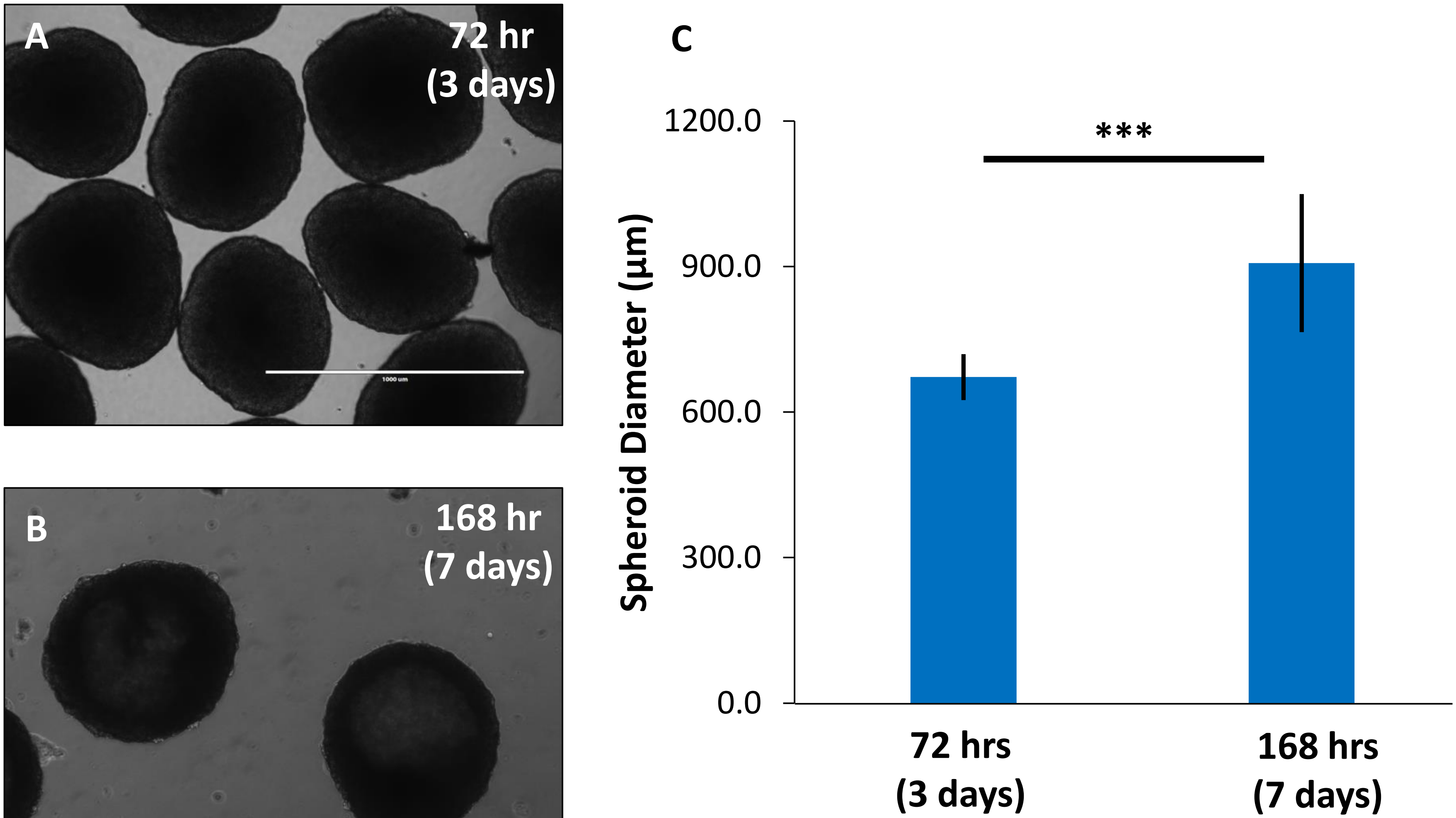
Ramped Rotation:

-15 rpm for 24 hrs

-5 rpm increase every 24 hrs up to 25 rpm

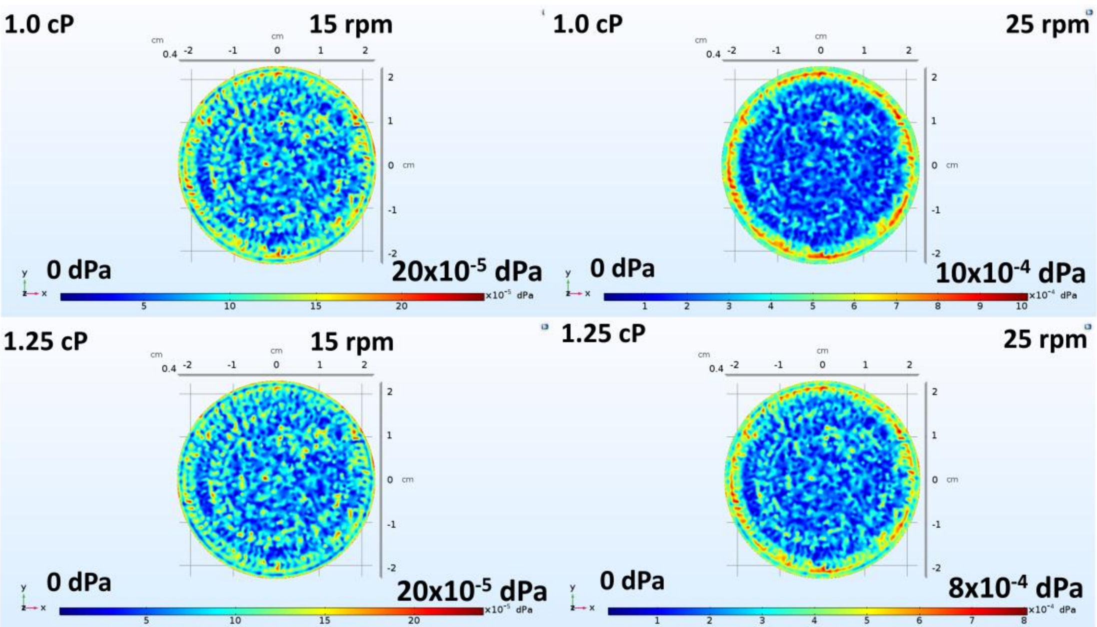
-72 hrs growth total

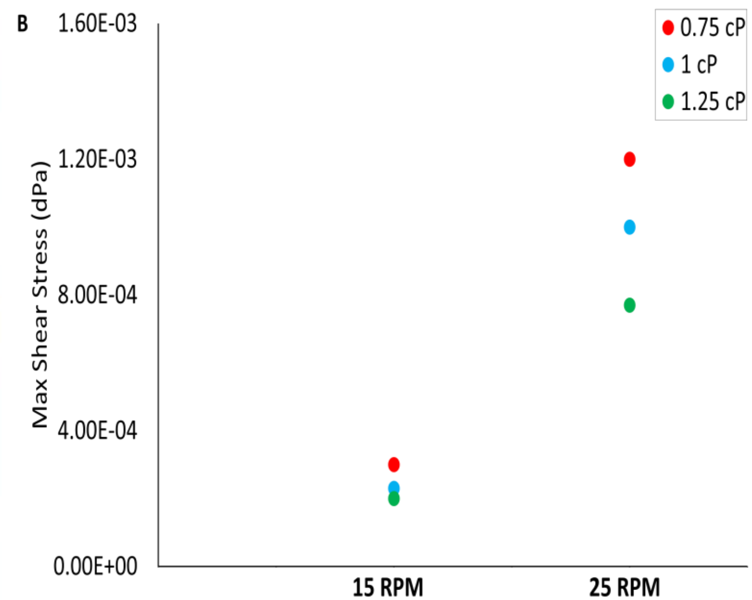
Spheroid Size Can Change Over Long-Term Culture



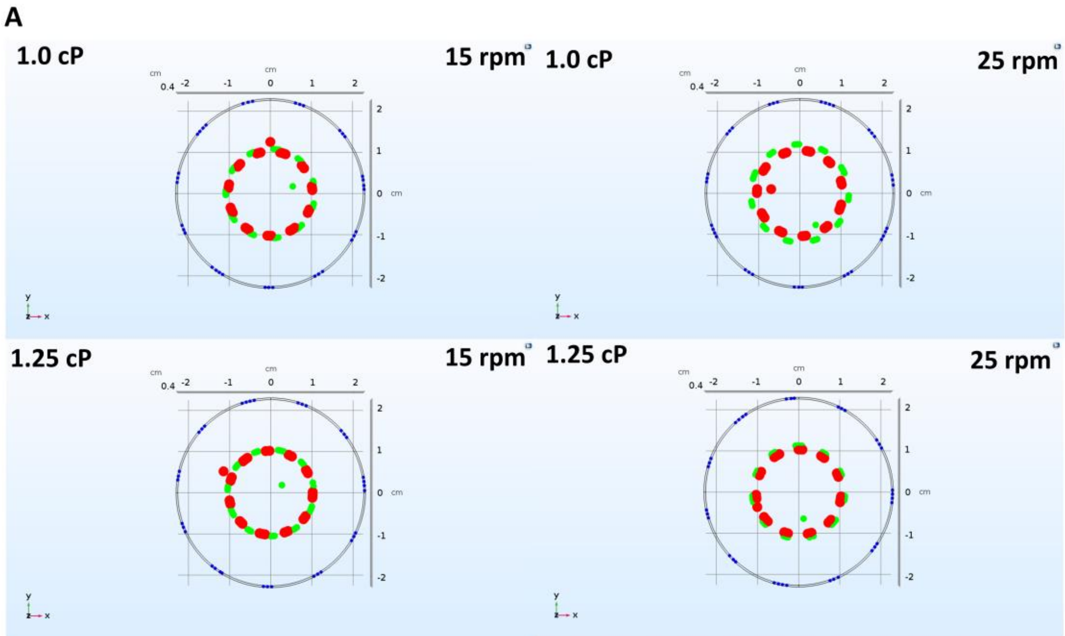
Scale Bar: 1000 µm

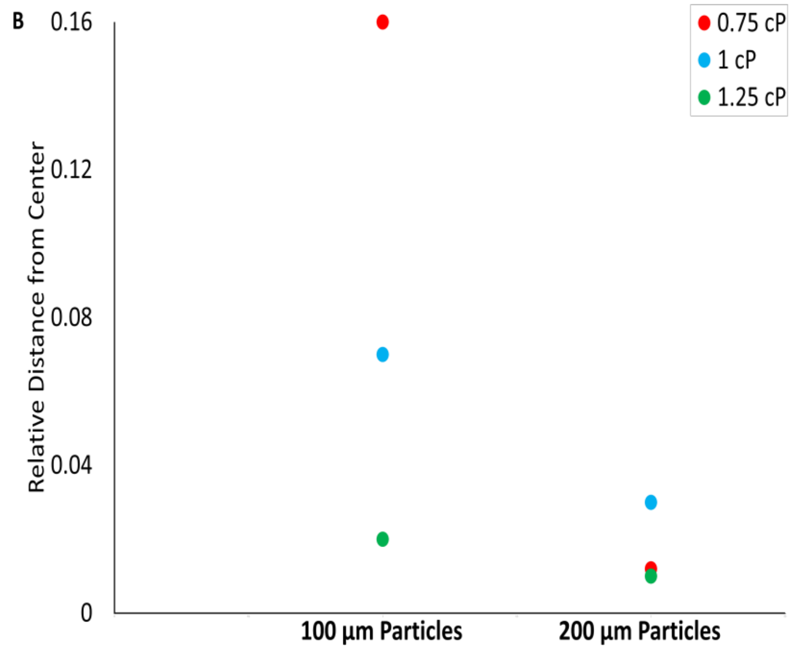
Increasing Viscosity Reduces Increase in Shear Stress at 25 rpm





Increasing Viscosity Restores Spheroids to Low-Shear Positions





Radius

- 50 µm
- 100 µm
- 200 µm

Summary/Future Directions

- CFD experiments show the low shear stress within HARVs. In addition, particle tracing models how the spheroids behave under that shear stress, with higher speeds pushing mid-sized spheroids towards the edge.
- To ensure consistency of shear stress across spheroid diameters, modulating the viscosity can align the spheroids closer to the middle, lowering the shear stress.
- This will be further verified and refined in the future with more simulations modeling the changes in shear stress over longer culture times as the spheroid size gradually changes.
- RCCS experiments with methylcellulose-supplemented media will also be used to verify the simulation results.

Acknowledgments

SUNY Poly

- Dr. James Castracane
- Dr. Andre Melendez
- Atul Dhall
- Logan Butt
- Matt Strohmayer
- Pujhitha Ramesh
- Tristen Head
- Dr. Madhu Hemachandra
- Dr. Natalya Tokranova

SpacePharma, Inc.

- Dr. Molly Mulligan
- Dr. Yair Glick

Funding

COLLEGES OF NANOSCALE SCIENCE + ENGINEERING SUNY POLYTECHNIC INSTITUTE

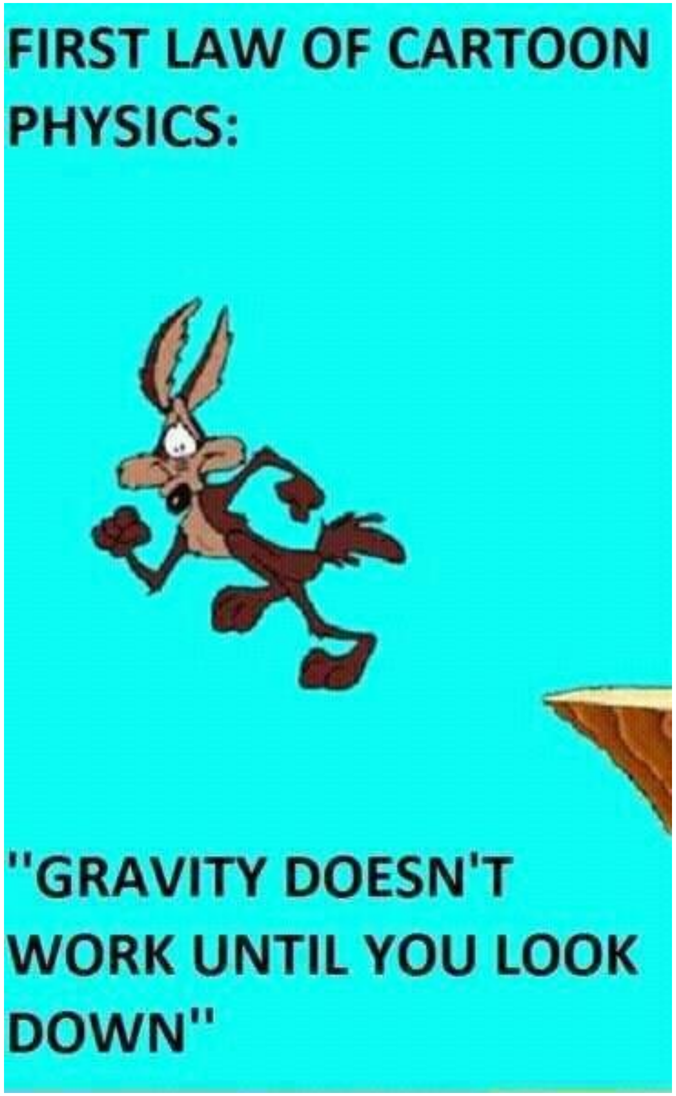
MATIMOP מתימו"פ
מרכז התעשיה הישראלית למחקר ופיתוח
Israeli Industry Center For R&D

References

- Becker JL, Souza GR. Using space-based investigations to inform cancer research on Earth. Nat Rev Cancer. 2013;13(5):315-27. doi: 10.1038/nrc3507. PubMed PMID: 23584334.
- Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL. Microbial responses to microgravity and other low-shear environments. Microbiol Mol Biol Rev. 2004;68(2):345-61. doi: 10.1128/MMBR.68.2.345-361.2004. PubMed PMID: 15187188; PMCID: PMC419922.
- Ebara M. Shape-memory surfaces for cell mechanobiology. Science and Technology of Advanced Materials. 2016;16(1):014804. doi: 10.1088/1468-6996/16/1/014804.
- Li Z, Cui Z. Three-dimensional perfused cell culture. Biotechnol Adv. 2014;32(2):243-54. doi: 10.1016/j.biotechadv.2013.10.006. PubMed PMID: 24184152.
- Cerwinka WH, Sharp SM, Boyan BD, Zhau HE, Chung LW, Yates C. Differentiation of human mesenchymal stem cell spheroids under microgravity conditions. Cell Regeneration. 2012;1(1):1.
- Vu, Lucas T., Rebekah R. Less, and Padmavathy Rajagopalan. "The promise of organotypic hepatic and gastrointestinal models." *Trends in biotechnology* 32.8 (2014): 406-413.
- Ma X, Pietsch J, Wehland M, Schulz H, Saar K, Hubner N, Bauer J, Braun M, Schwarzwalder A, Segerer J, Birlem M, Horn A, Hemmersbach R, Wasser K, Grosse J, Infanger M, Grimm D. Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. FASEB J. 2014;28(2):813-35. doi: 10.1096/fj.13-243287. PubMed PMID: 24196587.
- Barzegari A, Saei AA. An update to space biomedical research: tissue engineering in microgravity bioreactors. Bioimpacts. 2012;2(1):23-32. doi: 10.5681/bi.2012.003. PubMed PMID: 23678438; PMCID: PMC3648913.
- Hammond T, Hammond J. Optimized suspension culture: the rotating-wall vessel. American Journal of Physiology-Renal Physiology. 2001;281(1):F12-F25.

Thank You!

- Q&A



https://www.pinterest.com/pin/695735842404992552/

Supplemental Content

COMSOL Math Details

-Single Phase Flow (spf) studies under laminar conditions and based off the Navier-Stokes equation (Frozen Rotor). The size of the HARV was constant so the variables were the rotating speed and the viscosity of the media. Rotation speeds for the RCCS are 15 and 25 rpm and the rotating domain was defined in the clockwise direction along the z-axis to mimic the horizontal orientation of HARVs on the RCCS.

-For Particle Tracing, particle sizes were chosen based on both our own experimental results and what has been previously published. The particle mass was calculated based on the volume of the spheroid and an assumed density of 1.04 g/cm^3 [6].

Equation 1

$$\rho(u \cdot \nabla)u = \nabla \cdot [-\rho \mathrm{I} + \mu(\nabla u + (\nabla u)^T)] + F + \rho g$$

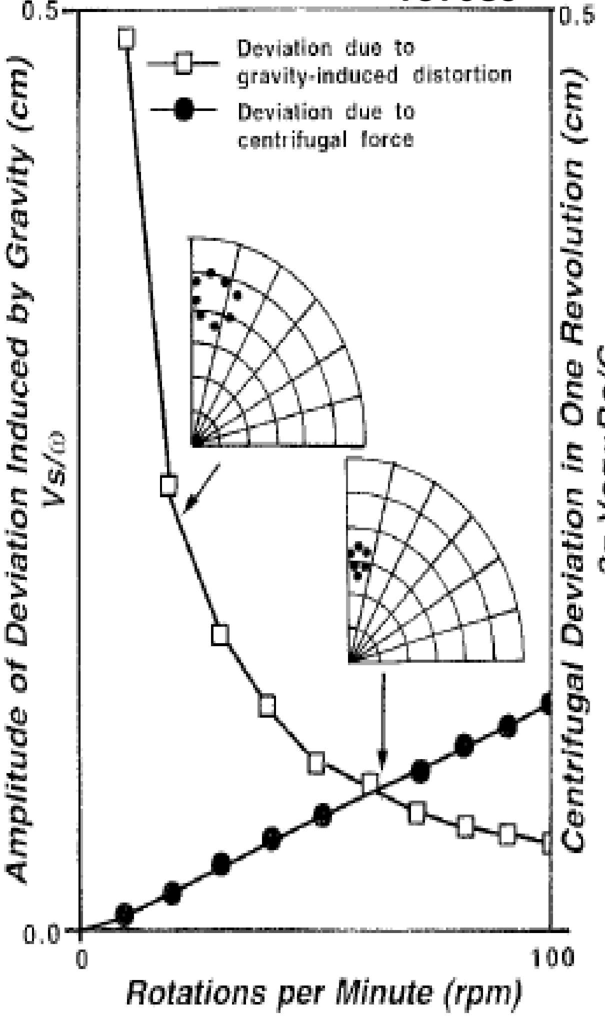
$$\rho \nabla \cdot (u) = 0$$

Equation 2 [3]

$$WSS = \sqrt{\tau^2{}_{xy} + \tau^2{}_{yz} + \tau^2{}_{xz}}$$

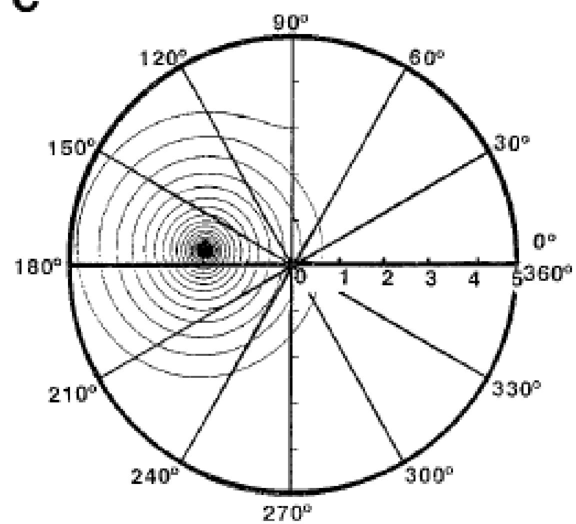
Balance of Coriolis and Centrifugal forces

B



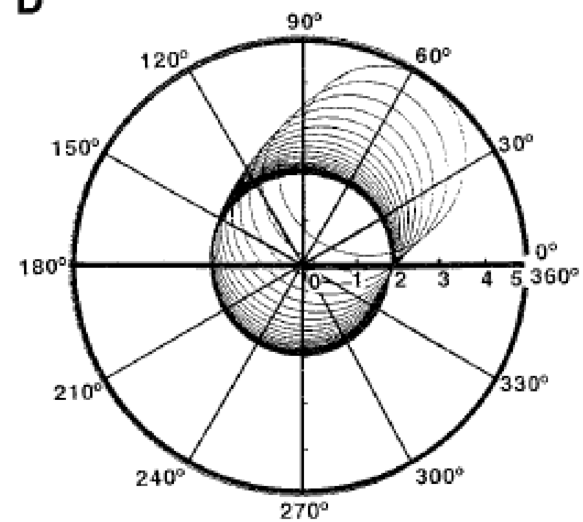
Hammond and Hammond, 2001

C



Particle Motion: Stationary Frame
-Achieves steady-state spiral balancing coriolis and centrifugal forces

D



Particle Motion: Rotating Frame
-Shows actual trajectory