Mechanotransductive regulation of gap-junction activity between MLO-Y4 osteocyte-like and MC3T3-E1 osteoblast-like cells in Three-Dimensional Co-Culture.

C.M. Juran[1], E.A. Blaber[1], E.A.C. Almeida[1].

[1]Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, USA

Presented by: Cassandra M. Juran, Ph.D.

ASGSR Annual Meeting October 28, 2016

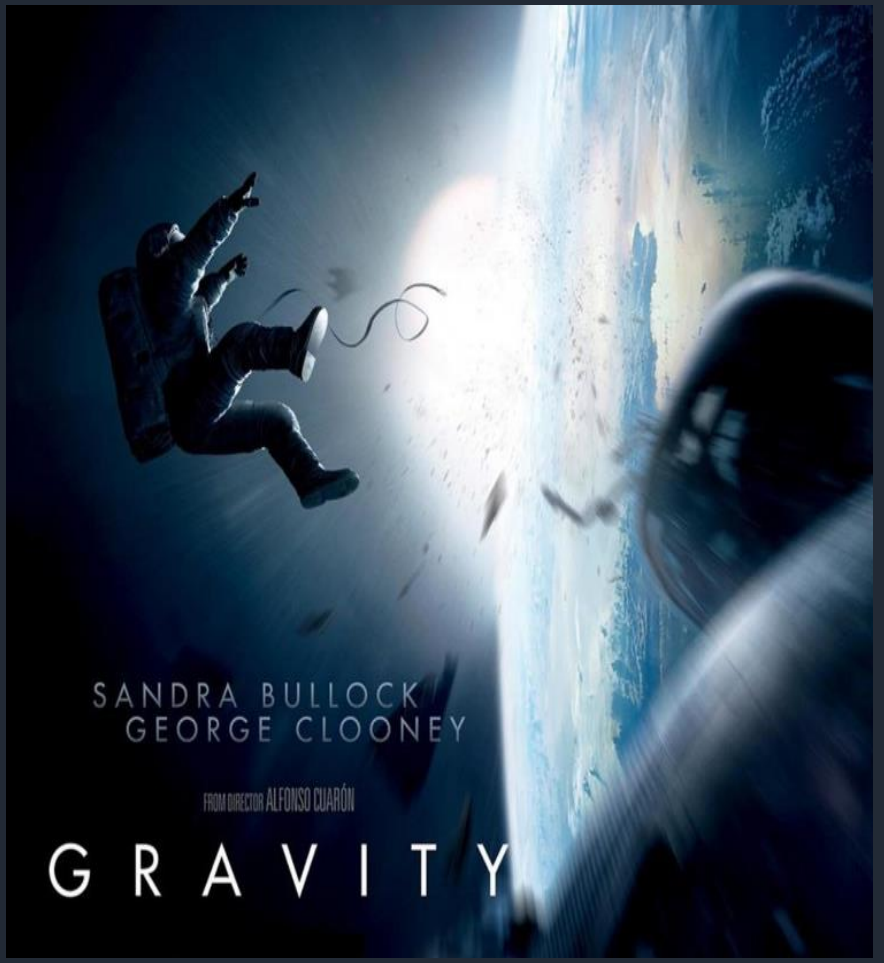
Space Biosciences

NASA AMES RESEARCH CENTER

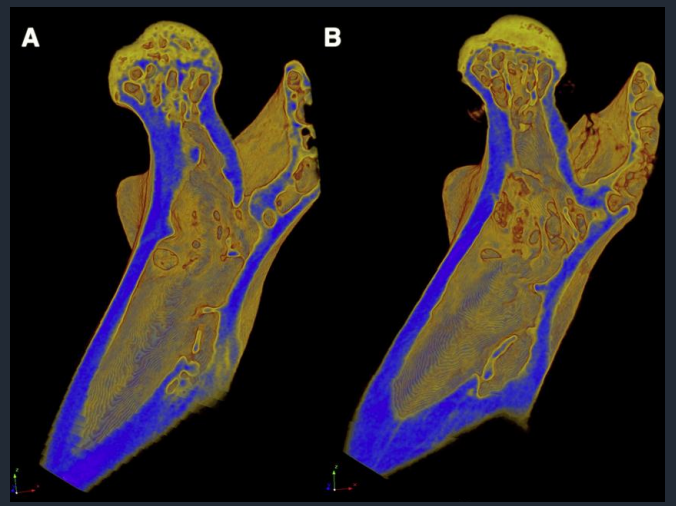
NASA POSTDCTORAL PROGRAM

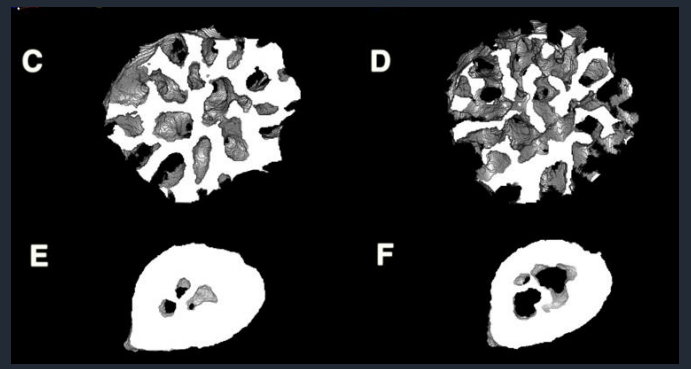
Unloading In Space

Research on these pioneering missions, aboard the Shuttle and currently the ISS have provided groundbreaking data illuminating the deleterious biological response of bone to mechanical unloading.



Even Hollywood is fascinated with Unloading

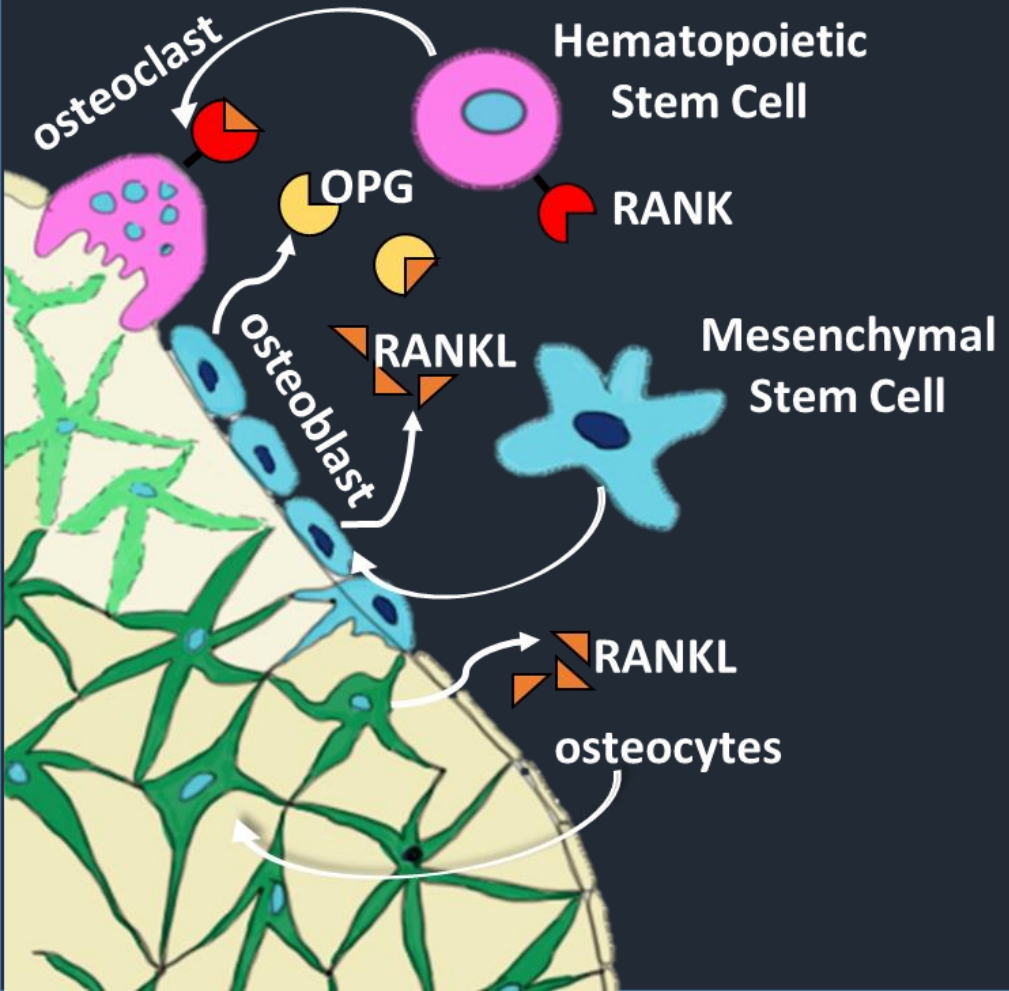




Blaber, E. A., et al. "Mechanical unloading of bone in microgravity reduces mesenchymal and hematopoietic stem cell-mediated tissue regeneration." *Stem cell research* 13.2 (2014): 181-201.

Balancing Act

- Skeletal System Homeostasis



Active Osteoclasts
Bone Resorption

Active Osteoblasts
Bone Formation

- Unloading interrupts the balance between bone resorption and bone formation
- But is that the whole story?

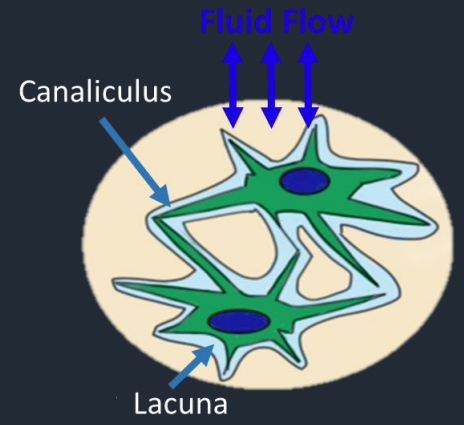
Our Investigation

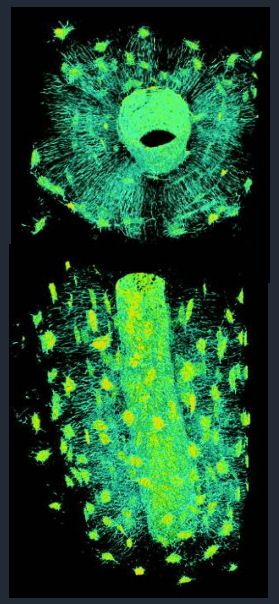
Stimulation → Gap Junction Activity → cell-cell communication → Regulation of Cell Activity

1. Are there regulatory actions of osteocytes only observable when direct communication via gap junctions with osteoblast are present in a physiologically relevant culture system?

2. Does the tissue analogous three dimensional orientation of the culture system and cellular population influence proliferative and recruitment phenotype expressions?

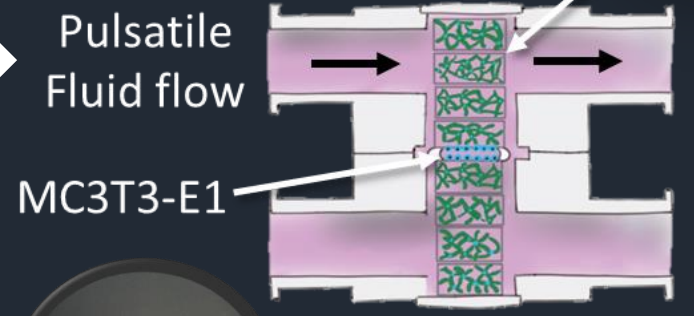
Physiologically Relevant Osteocyte/Osteoblast Organization

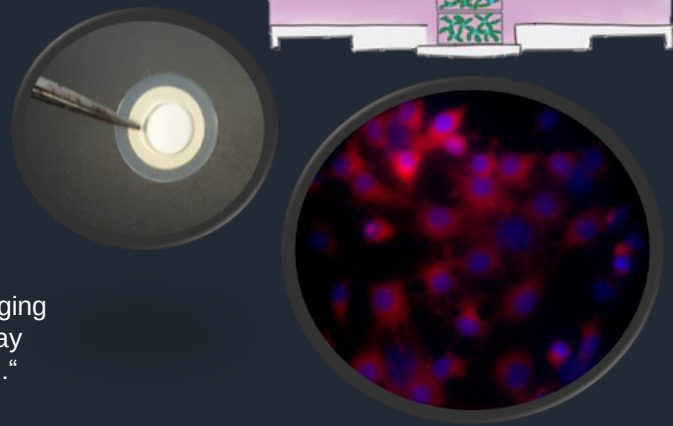




Longitudinal Compressive Load

Pacureanu, Alexandra, et al. "Nanoscale imaging of the bone cell network with synchrotron X-ray tomography: optimization of acquisition setup." *Medical physics* 39.4 (2012): 2229-2238.

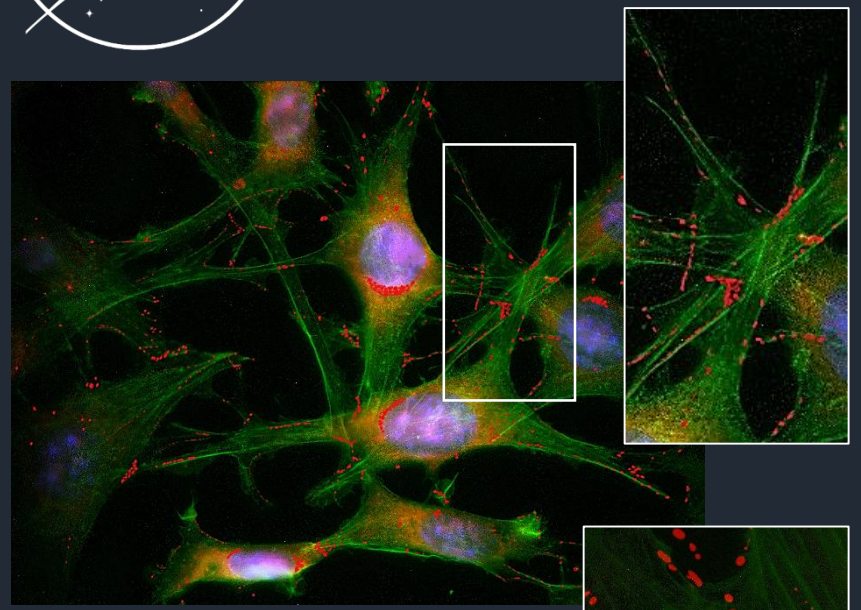


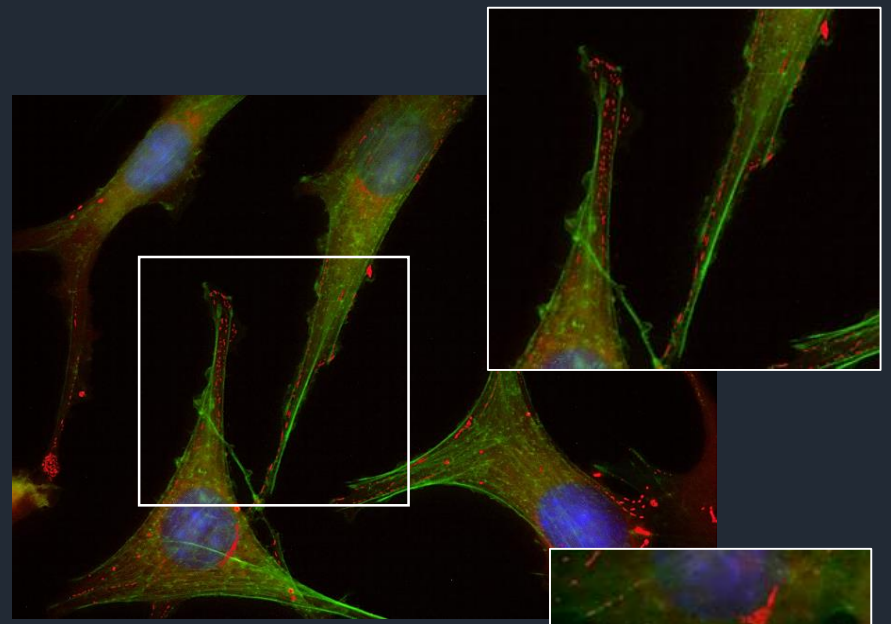


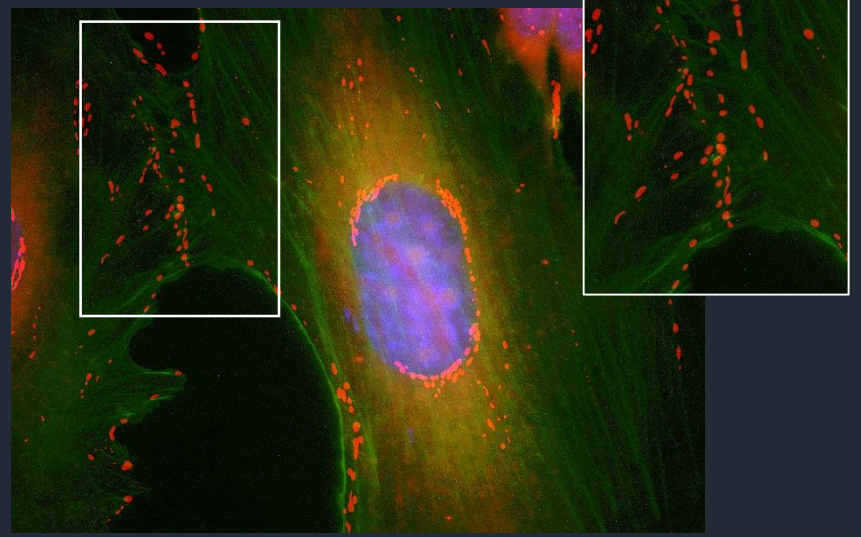
Environment Provides

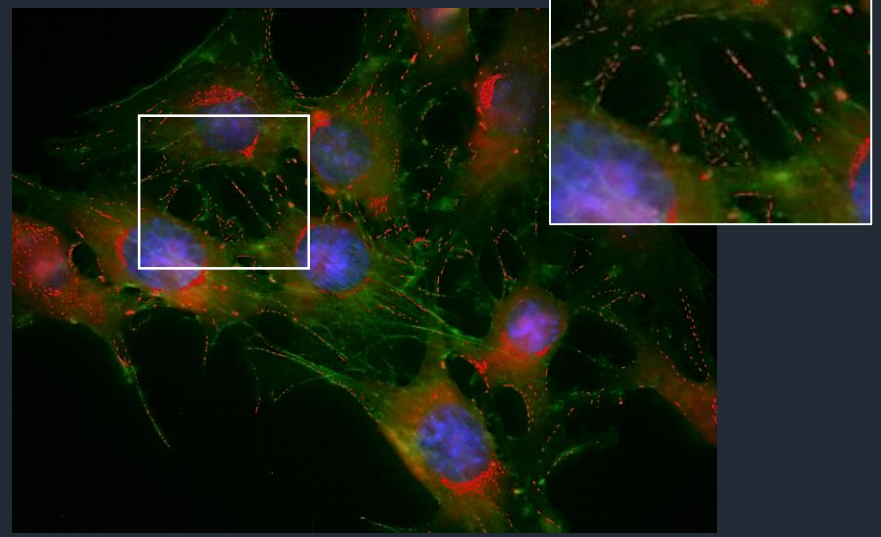
- **Cell-Cell contact** enabled by adjacent culture
- Three dimensional osteon shape and osteocyte orientation and **interconnectivity** represented
- Flow only experienced by the osteocyte culture
- **Imaging** conducted without disturbing the culture

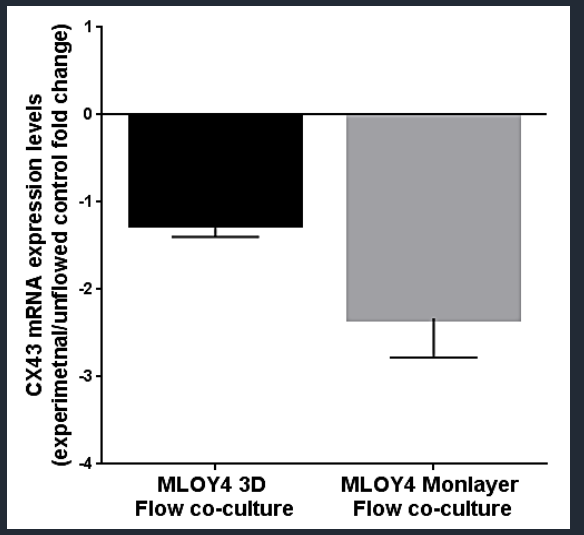
Connexin-43 Direct Communication Activity

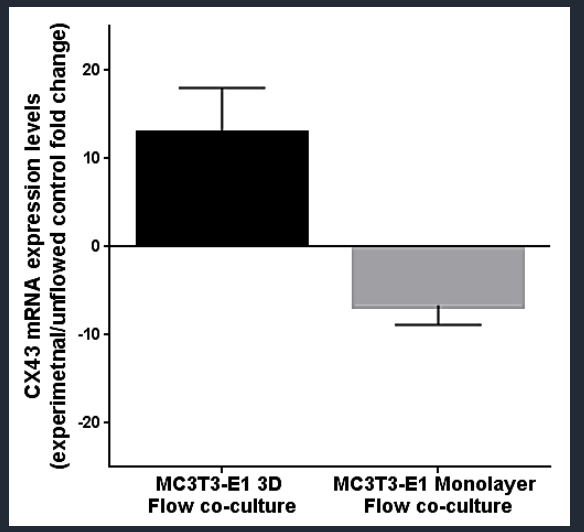




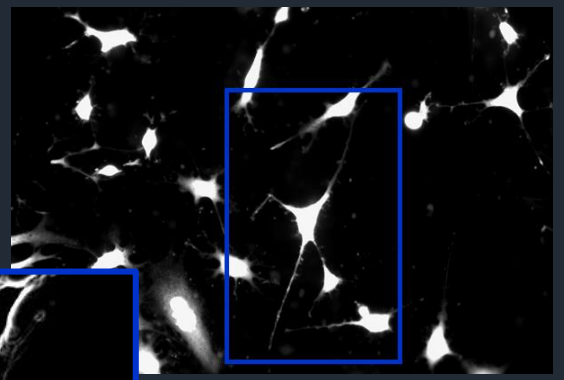


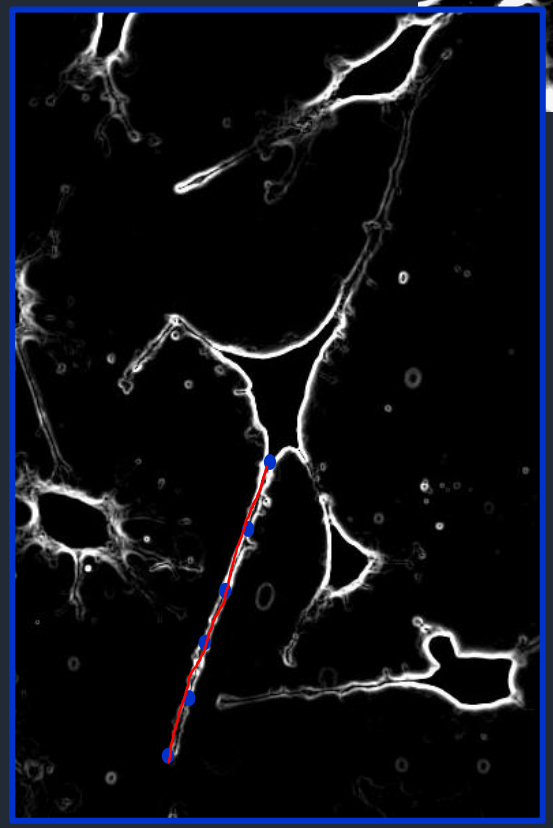


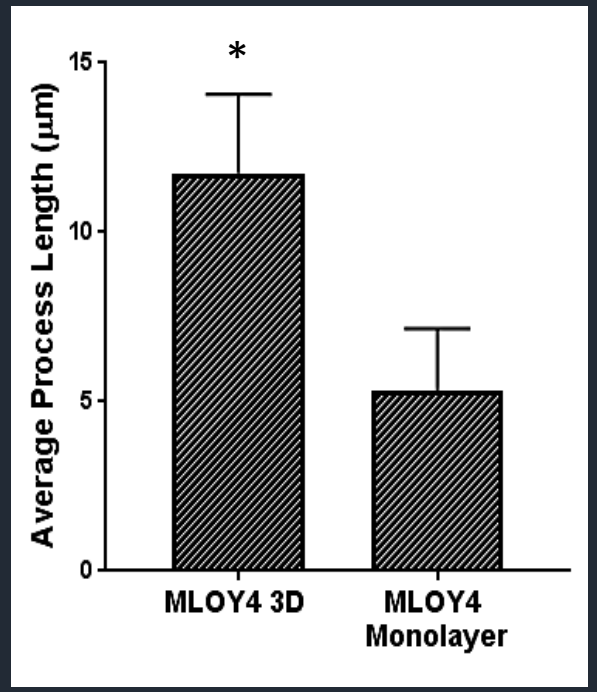


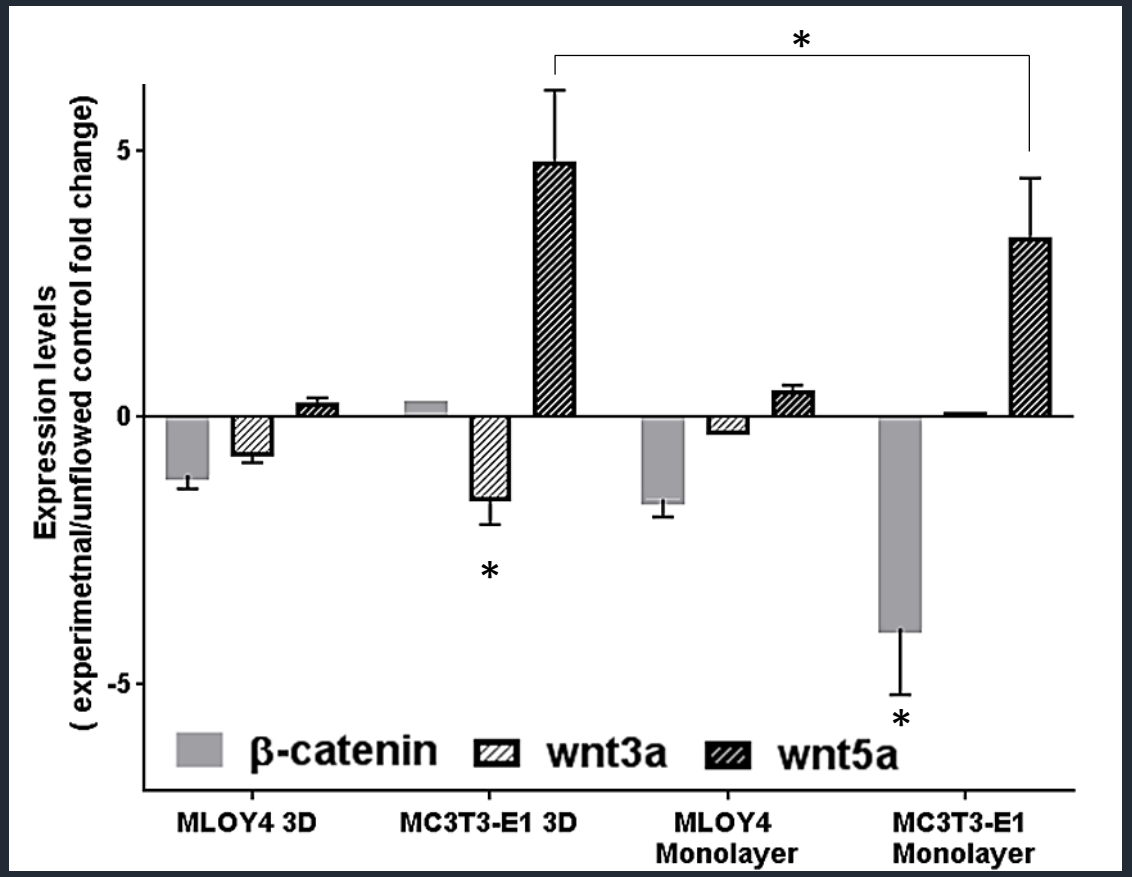


3D Organization effects on Example Osteogenic Signal Transduction Pathway



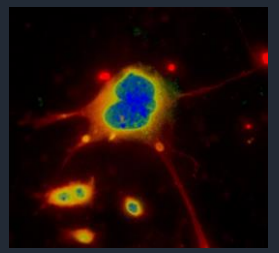


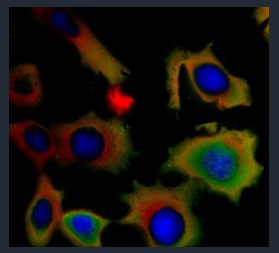


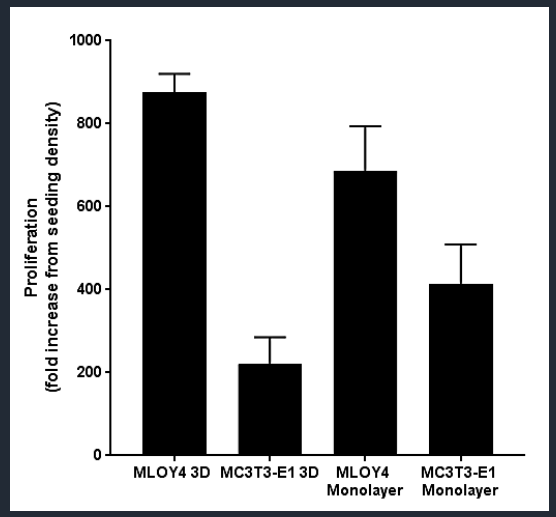


Proliferation Regulation and Osteoclastogenesis Niche Cell Recruitment

Yue Zhang[1], Emmanuel M.
Sundar Srinivasan[3], Ted S.
1 Division of Musculoskeletal Sciences, Depa
2 Department of Cellular and Molecular Ph
University of Washington, Seattle, Washing







MLOY4 3D

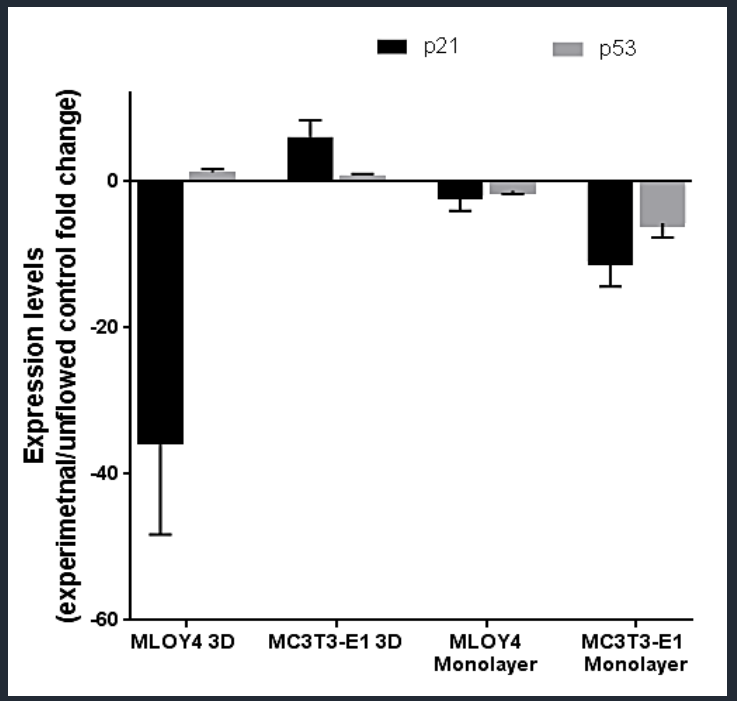
MC3T3-E1 3D

CX43

p21

Proliferation

RANKL/OPG



MLOY4 Monolayer

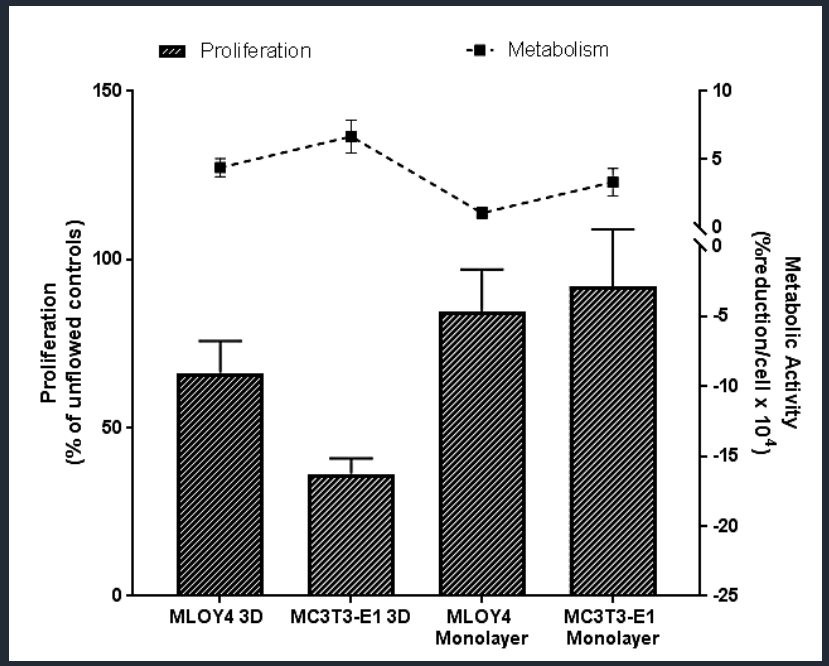
MC3T3-E1 Monolayer

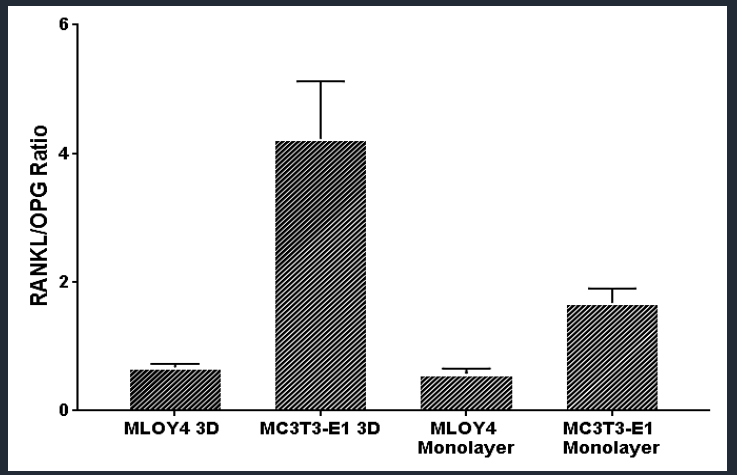
CX43

p21

Proliferation

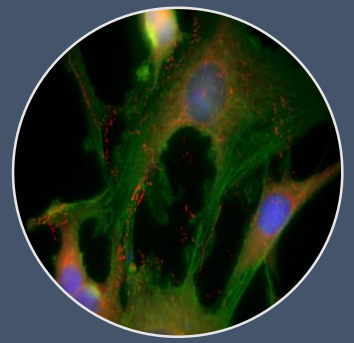
RANKL/OPG



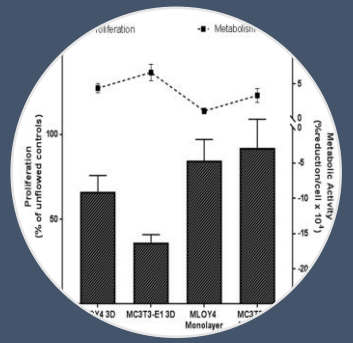


Direct Communication regulation may be propagating and summative

Physiologically relevant cell organization in co-culture.



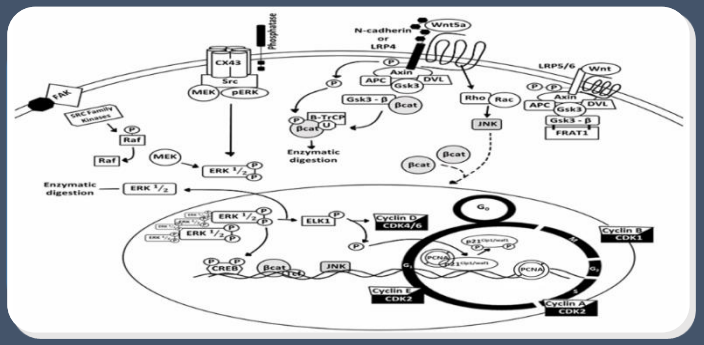
Importance of distribution of CX43 in response to mechanical loading.



Mechanical Regulation of Proliferative mechanisms via CX43 activity

Mechanical Regulation of molecular cell cycle behavior

Future Work



More fully defined osteoregulator and osteogenic molecular pathways

- Pathways of interest include
- Gap Junction Connexin 43
- Non-canonical Wnt (Wnt5a group – specifically Wnt11)
- PCNA (Proliferating cells nuclear antigen)

Acknowledgements

Eduardo Almeida, Ph.D.
Elizabeth Blaber, Ph.D.
Margareth Cheng-Campbell
Oscar Roque

NASA Space Biology Grant NNH14ZTT001N-0062
E. Blaber and NNH14ZTT001N-003 to E. Almeida