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

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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.

**Balancing Act**

- **Skeletal System Homeostasis**

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

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**Active Osteoclasts**

- Bone Resorption

**Active Osteoblasts**

- Bone Formation

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**Hematopoietic Stem Cell**

**Mesenchymal Stem Cell**

**OPG**

**RANK**

**RANKL**

**Osteoclast**

**Osteoblast**

**Osteocytes**
Our Investigation

Stimulation $\rightarrow$ Gap Junction Activity $\rightarrow$ cell-cell communication $\rightarrow$ 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

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

- **CX43**
  - MLOY4 3D
  - MC3T3-E1 3D
  - CX43 Monolayer
  - Monolayer

- **p21**
  - MLOY4 3D
  - MC3T3-E1 3D
  - MLOY4 Monolayer
  - MC3T3-E1 Monolayer

- **Proliferation**
  - MLOY4 3D
  - MC3T3-E1 3D
  - MLOY4 Monolayer
  - MC3T3-E1 Monolayer

- **RANKL/OPG**
  - MLOY4 3D
  - MC3T3-E1 3D
  - MLOY4 Monolayer
  - MC3T3-E1 Monolayer
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

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)
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