

Osteocytes Mechanosensing in NASA Rotating Wall Bioreactor



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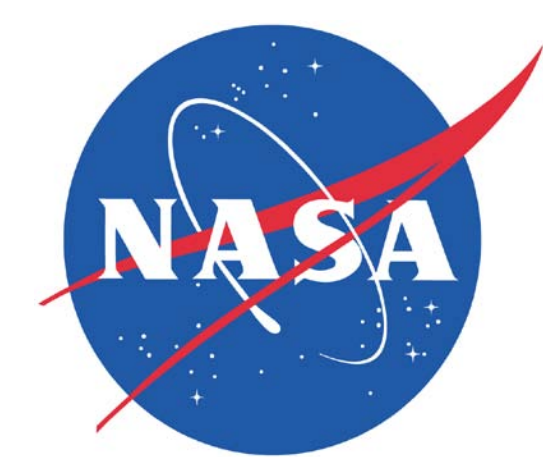
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INTRODUCTION

Osteocyte cells are the most abundant (90%) yet least understood bone cell type in the human body. Osteocytes are theorized to be the mechanosensors and transducers of mechanical load for bones, yet the biological mechanism of this action remains elusive. However, recent discoveries in osteocyte cell biology have shed light on their importance as key mechanosensing cells regulating bone remodeling and phosphate homeostasis.

The aim of this project was to characterize gene expression patterns and protein levels following exposure of MLO-Y4, a very well characterized murine osteocyte-like cell line, to simulated microgravity using the NASA Rotating Wall Vessel (RWV) Bioreactor.

To determine mechanistic pathways of the osteocyte's gravity sensing ability, we evaluated in vitro gene and protein expression of osteocytes exposed to simulated microgravity.

Improved understanding of the fundamental mechanisms of mechanotransduction at the osteocyte cellular level may lead to revolutionary treatment options to mitigate the effects of bone loss encountered by astronauts on long duration space missions and provide tailored treatment options for maintaining bone strength of immobilized/partially paralyzed patients here on Earth.

BACKGROUND

- **Osteocytes**
- Osteocytes are terminally differentiated osteoblasts embedded in the bone matrix
- Roles in mechanosensation, and possible role in phosphate homeostasis
- Osteocyte ablation results in osteoporosis (Tatsumi et al. 2007)
- **Sclerostin/SOST & Osteocytes**
- Osteocytes control osteoblast differentiation and activation through the Sclerostin-Wnt signaling pathway
- **Future Targeted Treatment Options:** Mature osteocytes are the only cells in the adult human known to express sclerostin, the protein product of the gene SOST.
- Hindlimb unloading in mice dramatically upregulates SOST/Sclerostin expression by osteocytes (Robling et al., 2008)
- SOST^{-/-} null mice are fully resistant to hindlimb induced bone loss (Lin, C, et al., 2009)
- PTH suppresses SOST expression (Bellido et al., 2005)

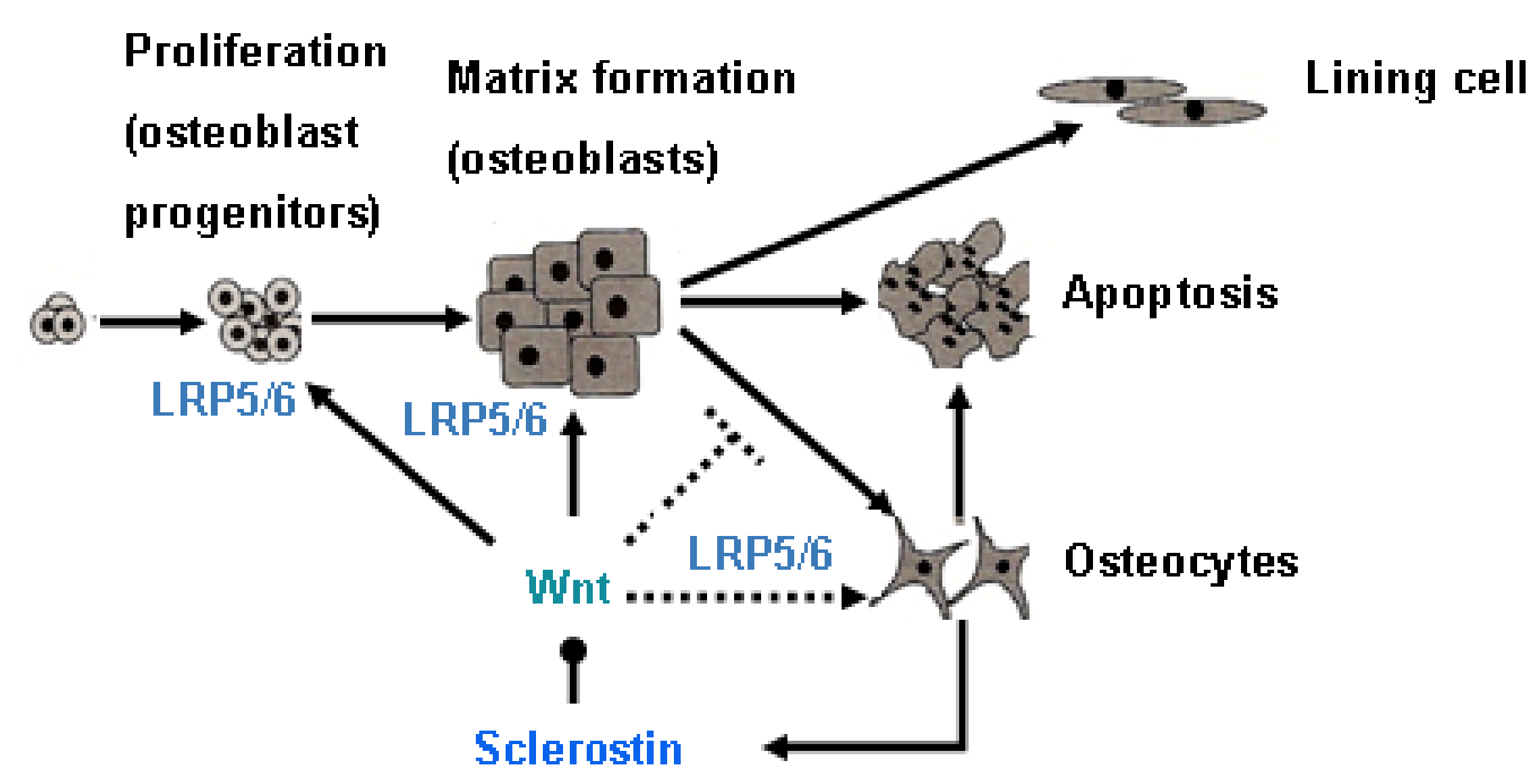


Figure 1: Sclerostin Mechanism of Action: Differentiation and osteoblasts activation mediated by Sclerostin/Wnt signaling pathway (adapted from Bilezikian et al., 2002)

MATERIAL & METHODS

Cells. MLO-Y4 cells were grown to confluence, harvested and seeded onto 3D collagen scaffold (BD) at a concentration of 250,000 cells/scaffold. Scaffolds (4 per condition) were either rotated in a vertical 50ml NASA/bioreactor vessel at a speed of 18 rpm (simulated microgravity), cultured in a horizontal 50 ml NASA bioreactor vessel at a speed of 18 rpm (control for the increased sheared environment of rotating vessel), or cultured into a static T-75 cm dish (static condition) at the laboratories of the National Space and Aeronautics Administration (NASA) Johnson Spaceflight Center (JSC).

Histology and immunohistochemistry. Scaffolds were either frozen with OCT or fixed in 10% formalin/PBS solution at 4 C ON, sectioned, processed and stained. Sections were stained with H&E or used for immunohistochemistry

Quantitative RT-PCR

Reverse transcription was performed on 1 µg of total RNA and semi quantitative RT-PCR was performed using the QuantiTect SYBR Green PCR Kit (QIAGEN) and the DNA Engine Opticon 2 qPCR system (MJ Research Inc.) according to the manufacturers' instructions.

Experimental Protocol (7, 14, 21 days)

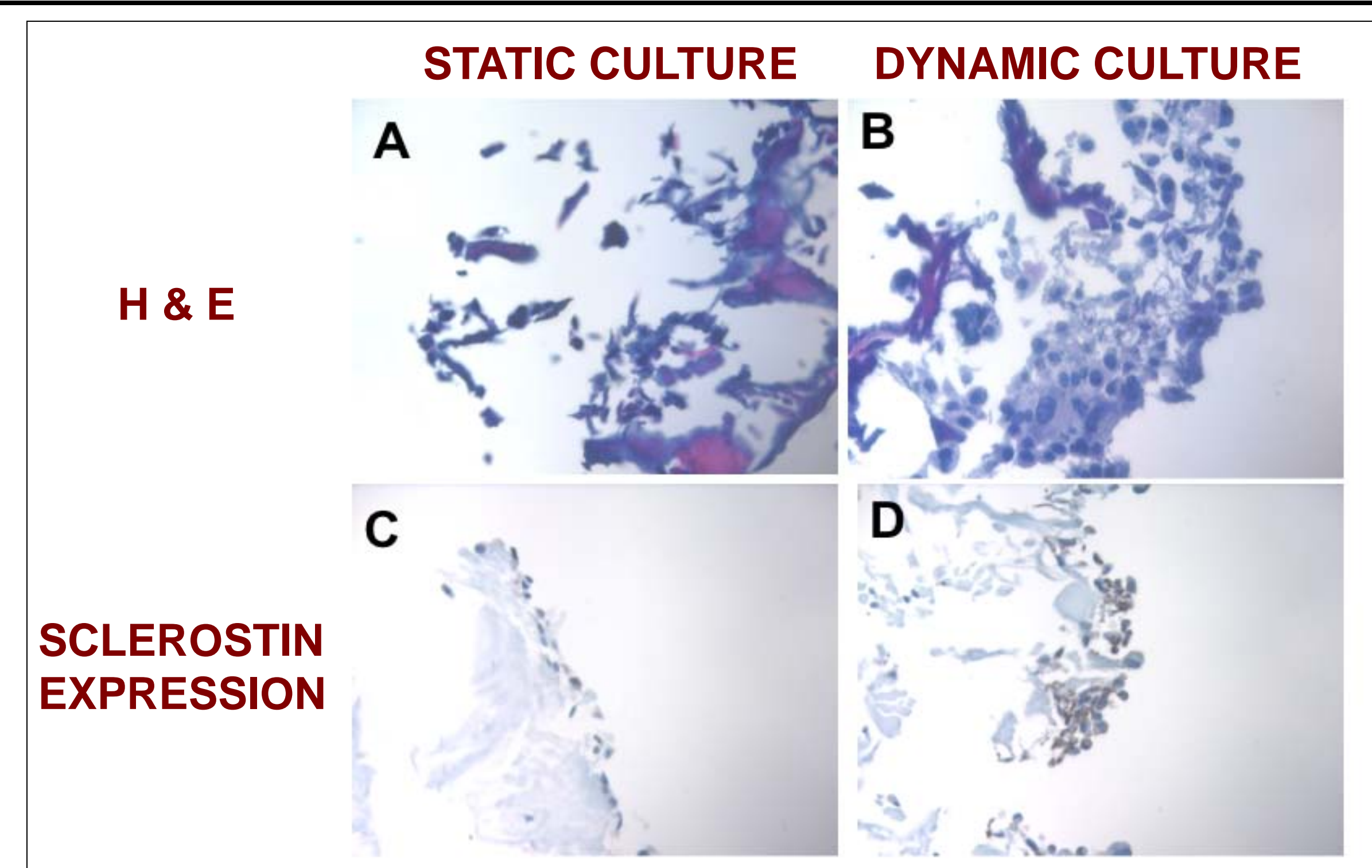
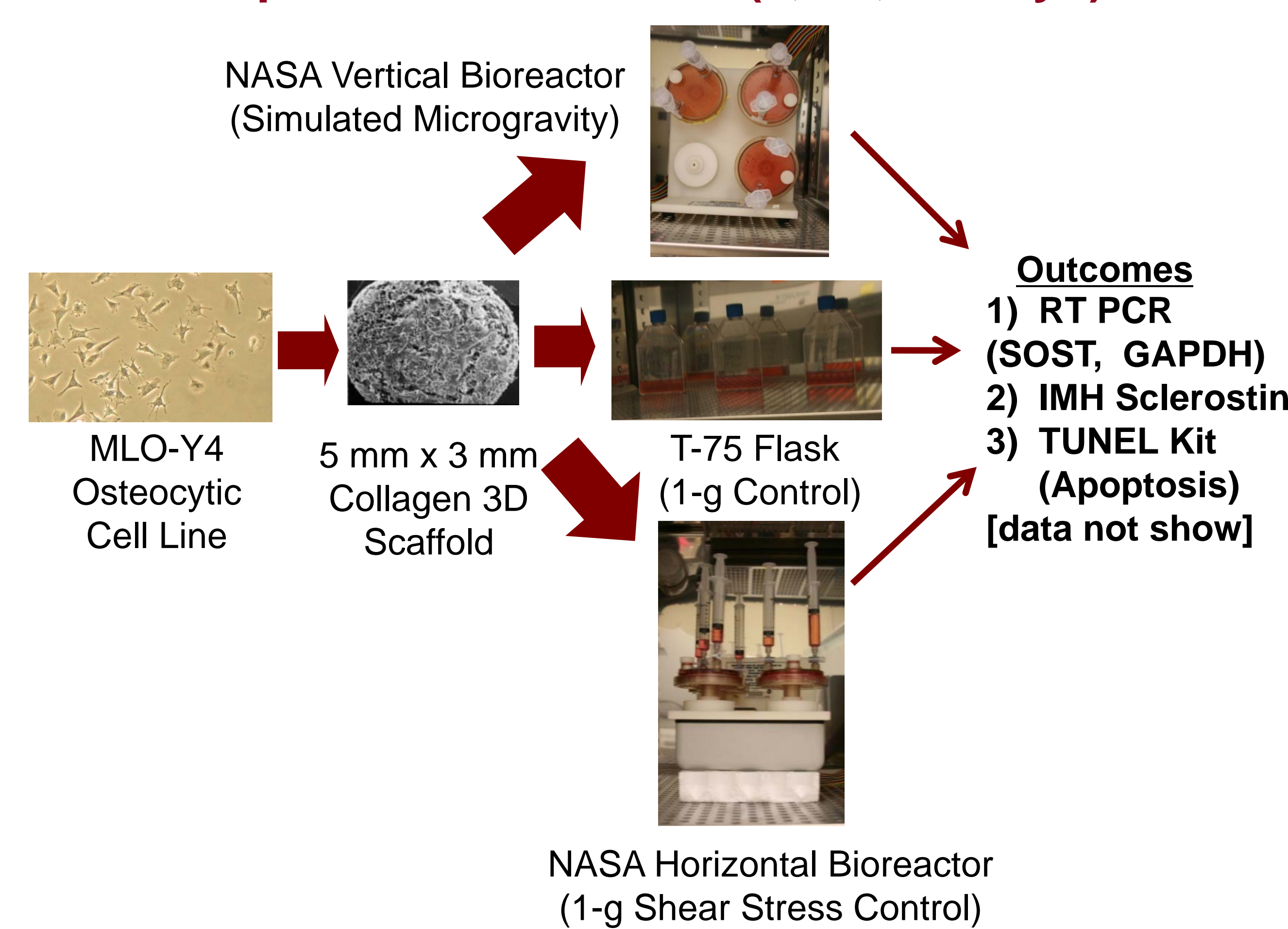


Figure 2: MLO-Y4 Osteocytic cells subjected to simulated microgravity: MLO-Y4 cells were grown on 3D collagen scaffold and subjected to static (A and C) or dynamic (B and D) culture conditions. H&E staining (A and B) showed increase in cell proliferation in MLO-Y4 cultured under dynamic vertical bioreactor condition (rotating bioreactor, panel B). Immunohistochemistry for Sclerostin (C and D) showed an increase in Sclerostin expression (black staining) in MLO-Y4 grown under dynamic vertical (simulated microgravity) bioreactor condition (panel D).

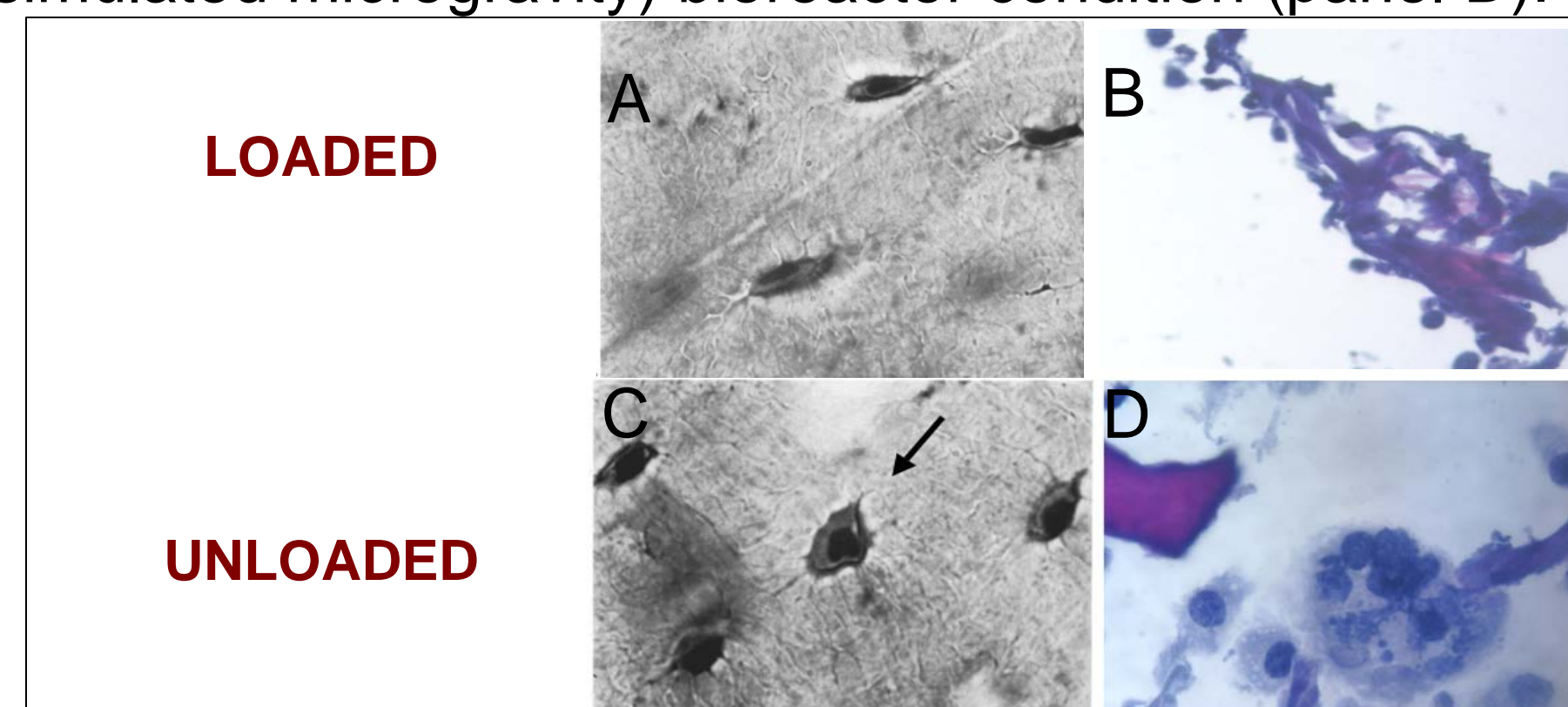


Figure 3: Unloading causes characteristic osteocyte morphologic changes: Osteocytes from immobilized rat (A and C, Krempien, 1976) and MLO-Y4 cultured on 3D collagen scaffold under simulated microgravity (B and D, H&E staining). Loaded osteocytes are spindle-shaped with small nuclei; unloaded osteocytes have enlarged round nuclei and cytoplasm.

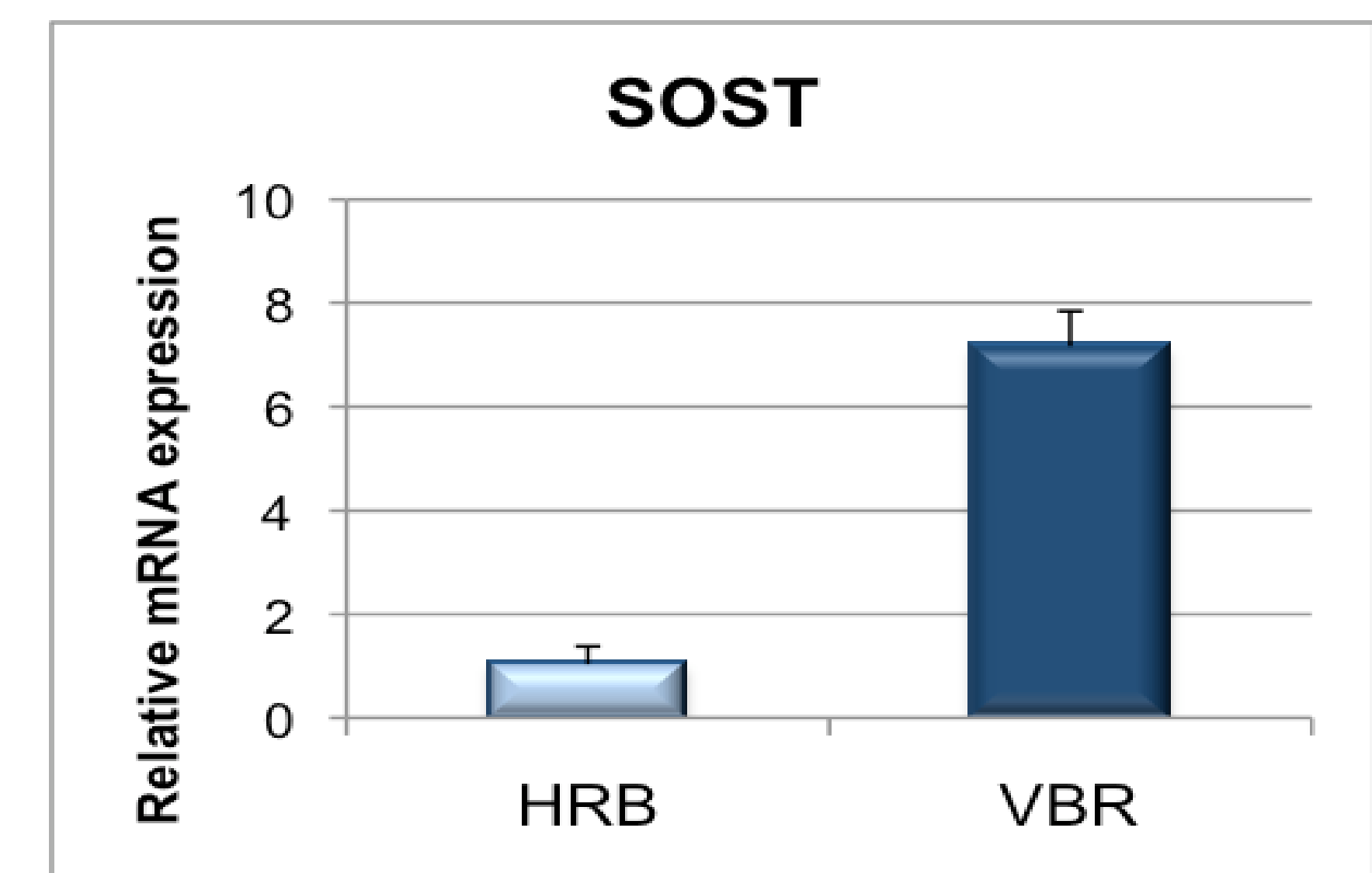


Figure 4: Simulated microgravity increases SOST expression @ 7 days: Real-time qPCR for SOST mRNA in MLO-Y4 cells cultured with mechanical stimulation alone (HRB) or in combination with simulated microgravity (VBR) for 7 days. Stimulated microgravity induced a 7 fold increase in SOST mRNA expression. Results are expressed as relative RNA and are normalized by GAPDH. Data are expressed as mean ± SD of triplicates. Experiments were repeated twice.

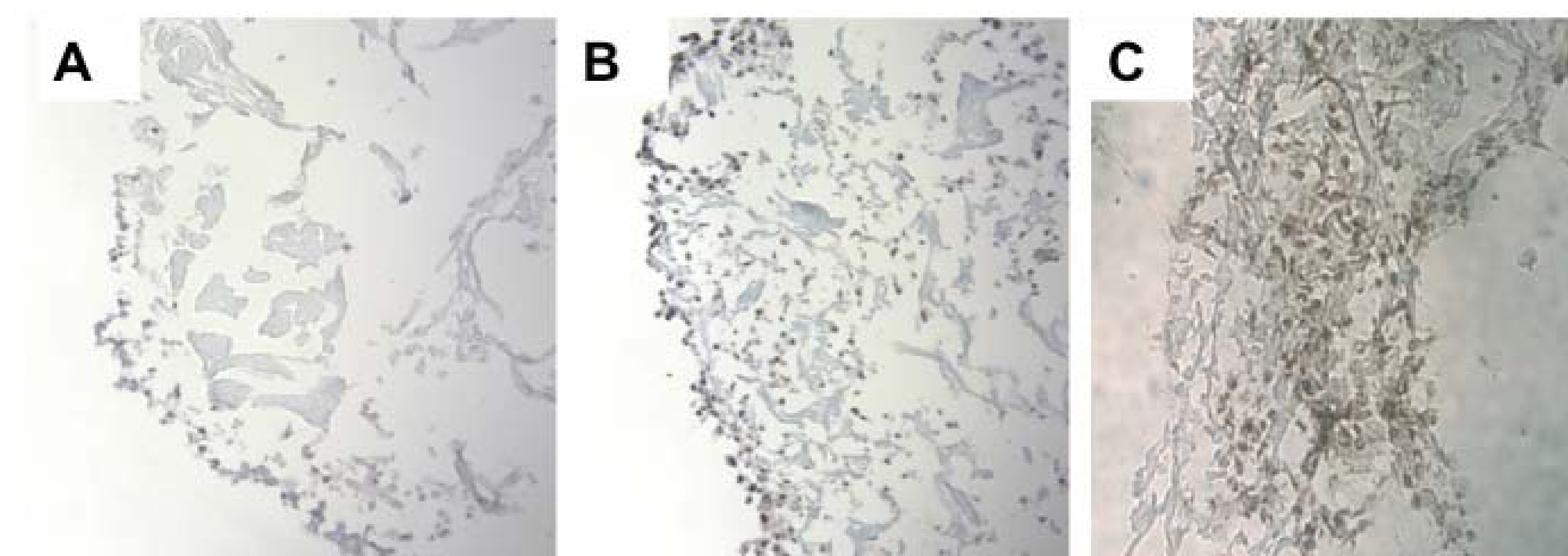


Figure 5: Sclerostin protein expression up-regulated @ 21 days: Sclerostin expression in MLO-Y4 cultured on 3D scaffold for 21 days under static (A), increased sheared environment (horizontal rotating bioreactor), (B), or simulated microgravity (vertical rotating bioreactor), (C).

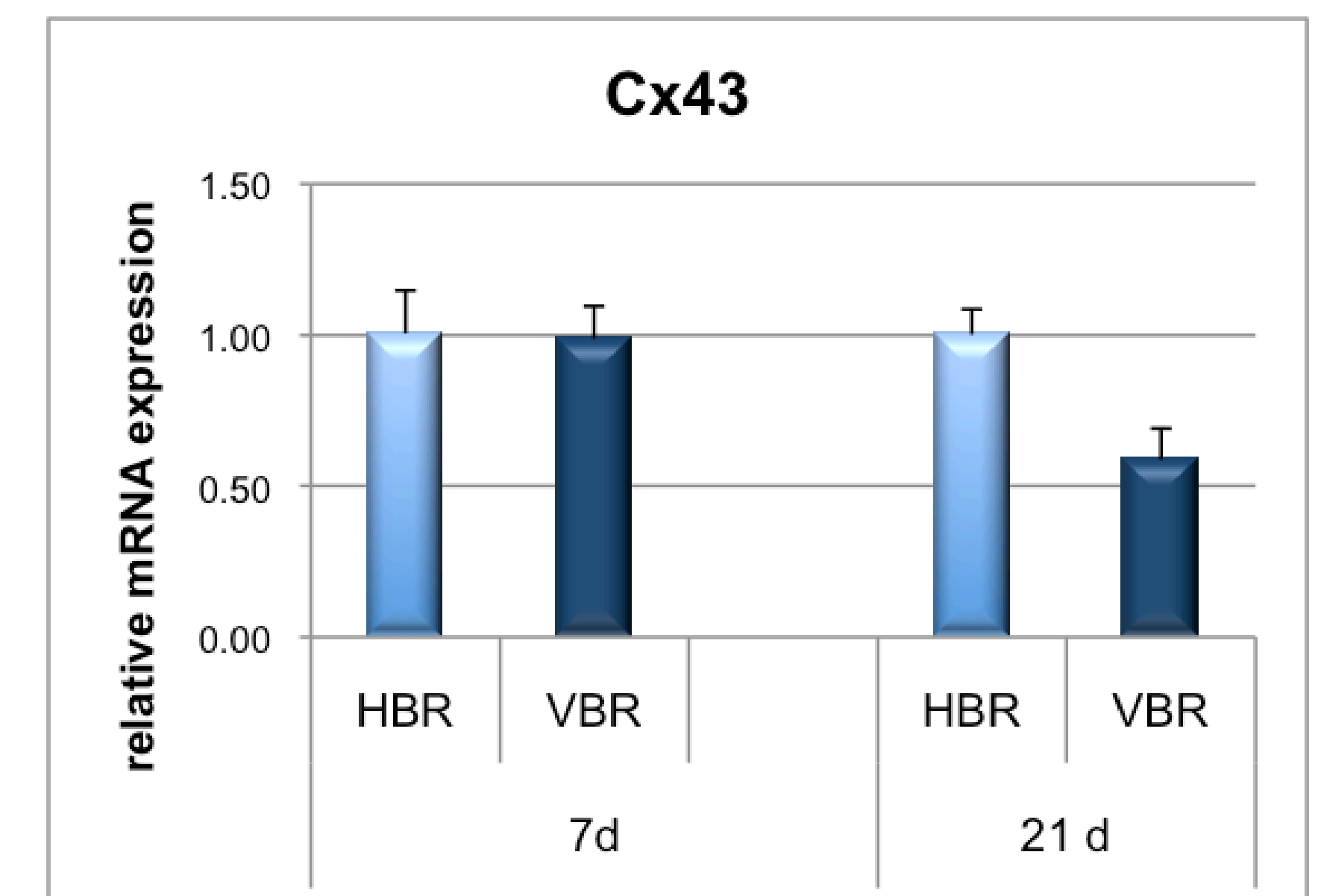


Figure 6: Simulated microgravity decreases Cnx43 expression @ 21 days: Real-time qPCR for Cnx43 mRNA in MLO-Y4 cells cultured with mechanical stimulation alone (HBR) or in combination with simulated microgravity (VBR) for 7 & 21 days. Stimulated microgravity induced a 40% decrease in Cnx43 mRNA expression. Results are expressed as relative RNA and are normalized by GAPDH. Mechanical stimulation has been shown previously to increase Cnx43 expression. Data are expressed as mean ± SD of triplicates. Experiments were repeated twice

Conclusions

- Simulated Microgravity in-vitro causes:
 - Osteocyte morphologic changes similar to immobilization unloading
 - Increases in SOST & Sclerostin expression relative to rotating control
 - Decreases Connexin-43 expression
- NASA/RWV mimics unloading to produce predictable regulation of known osteocyte mechanotransduction pathways at the molecular-cellular level. Future flight studies onboard ISS are needed for validation.
- NASA/RWV in-vitro osteocyte model allows future studies to investigate mechanotransduction pathways difficult to impossible to be conducted in-vivo

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