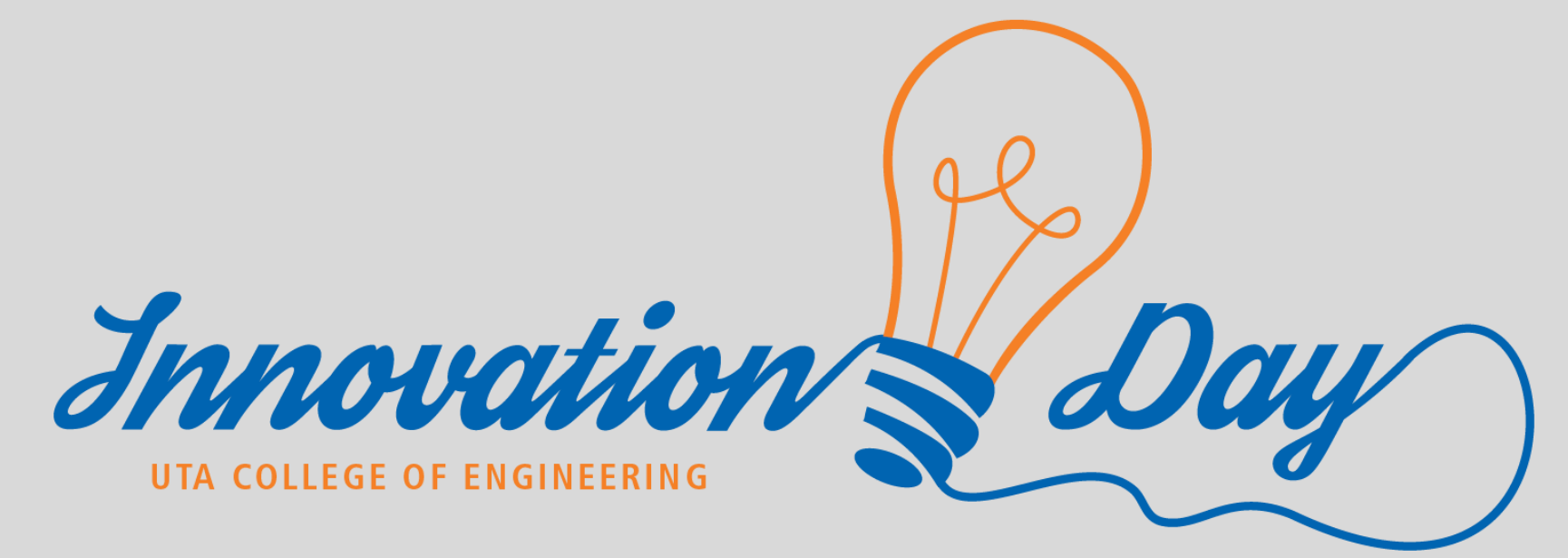


Adipose-Derived Stem Cell Osteogenic Differentiation Mediated by Photobiomodulation (1064nm)

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Background

Introduction

- Adipose-derived stem cells (ADSCs) are multipotent stem cells that have been routinely utilized.
- ADSCs prefer to differentiate into adipose tissue, but can differentiate into other cell types like bone, cartilage, and muscle.
- Photobiomodulation (PBM) is the use of low-level infrared light to stimulate biological processes.
- PBM has been shown to slow adipogenesis in ADSCs.

Hypothesis

- PBM treatment increases the efficiency of bone cell formation, even though the cell type was intentionally mismatched, and osteogenesis is mediated and facilitated by the metabolic activity of ADSCs.

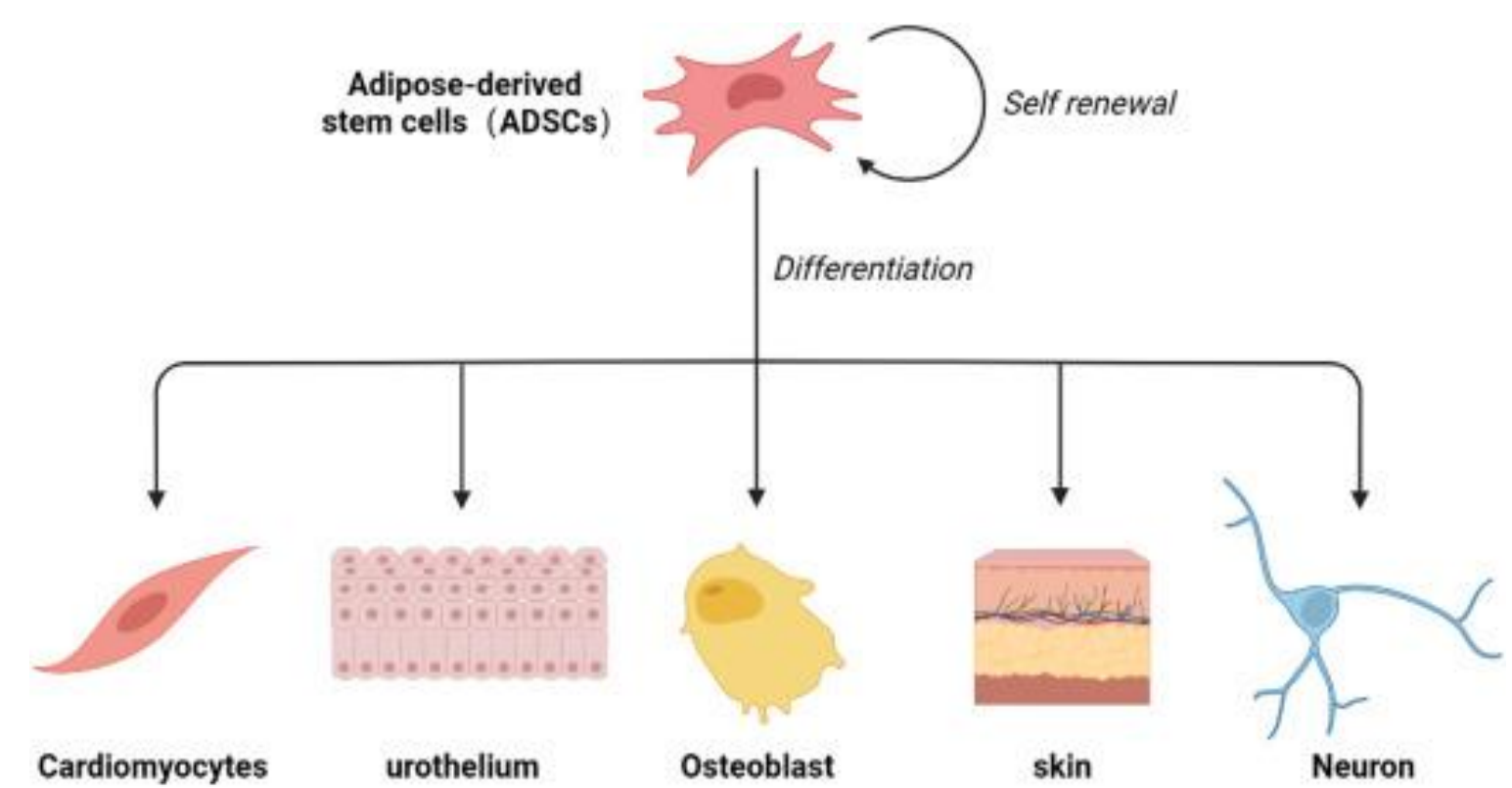


Figure 1. ADSCs can differentiate into many cell types.

Methods

Cell Culture

- When cells are confluent, split into a 48-well plate.
- Seed the cells at 17,000 cells per well.
- Allow cells to grow in growth media for 24 hours.

Stem Cell Differentiation

- After 24 hours, change media to osteogenic differentiating media.
- Continuing to change media every 3 days until the experiment is over.

Laser Treatment

- Fluence(J/cm²)= $\frac{W \times t}{A}$
- Low PBM Treatment
 - 2 watts
 - 30 seconds
 - ➔ 4.4 J/cm²
- High PBM Treatment
 - 2 watts
 - 30 seconds
 - ➔ 17.6 J/cm²
- Cells were treated for 7 days.

Results

Alizarin Red

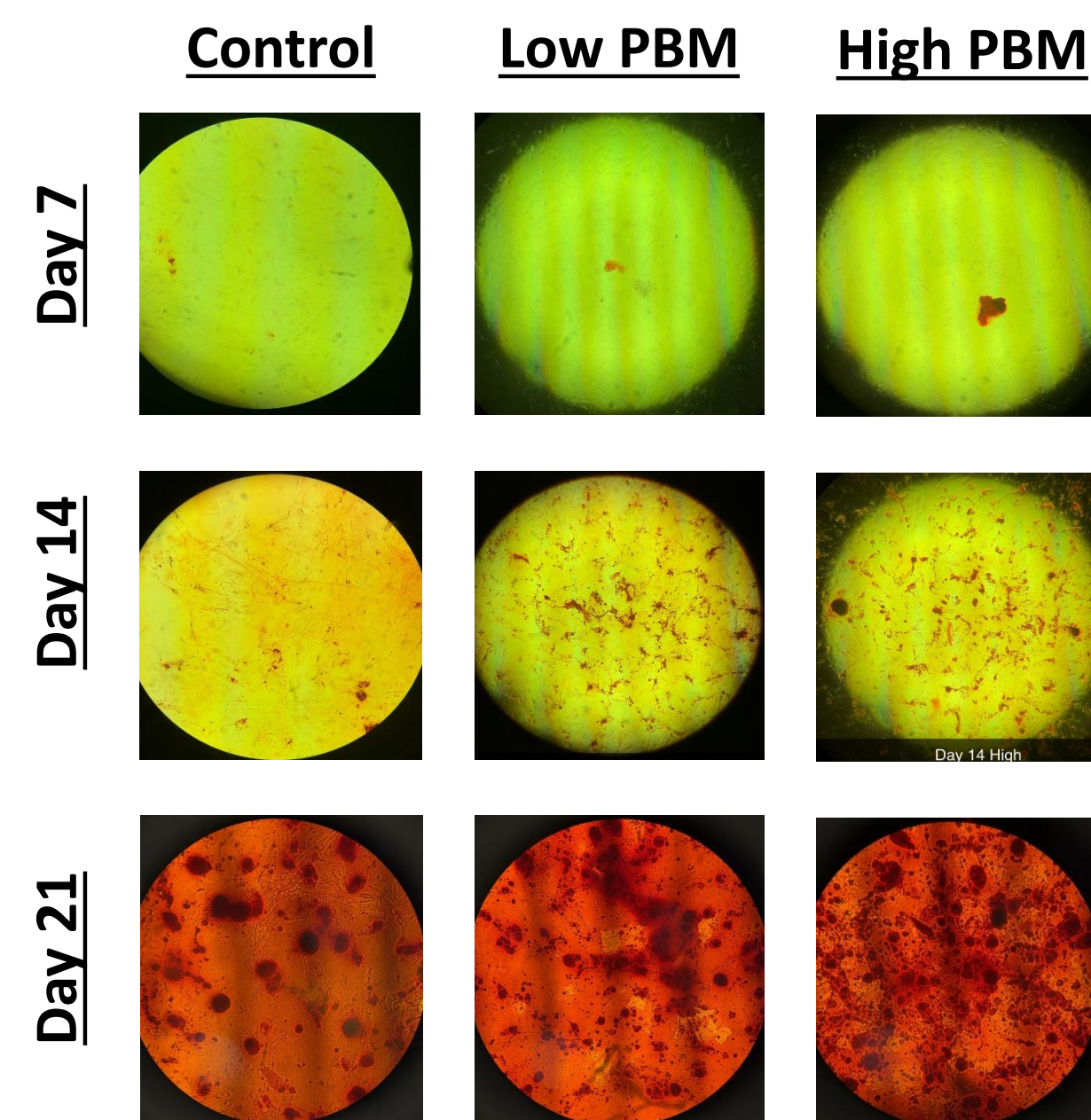


Figure 2. ADSCs in control, low PBM, and high PBM treatments at days 7, 14, and 21. Alizarin Red is a dye used to detect calcium deposits and to visualize mineralization as a marker for osteogenic differentiation.

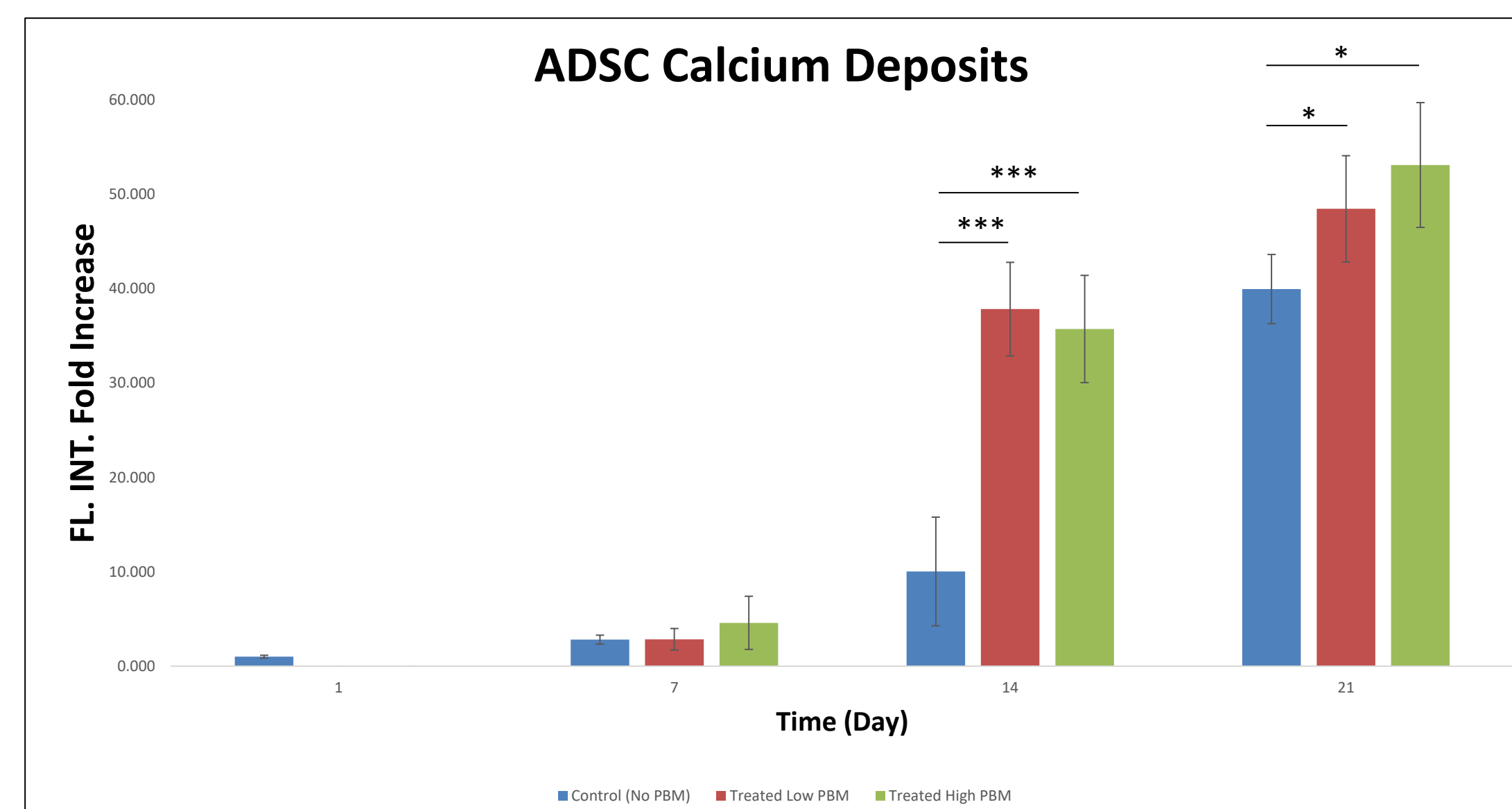


Figure 3. Quantification of the calcium deposits produced by ADSCs in control osteogenesis and treated with a low or high PBM at days 7, 14, and 21. * p<0.05 and *** p<0.001.

MTT Assay

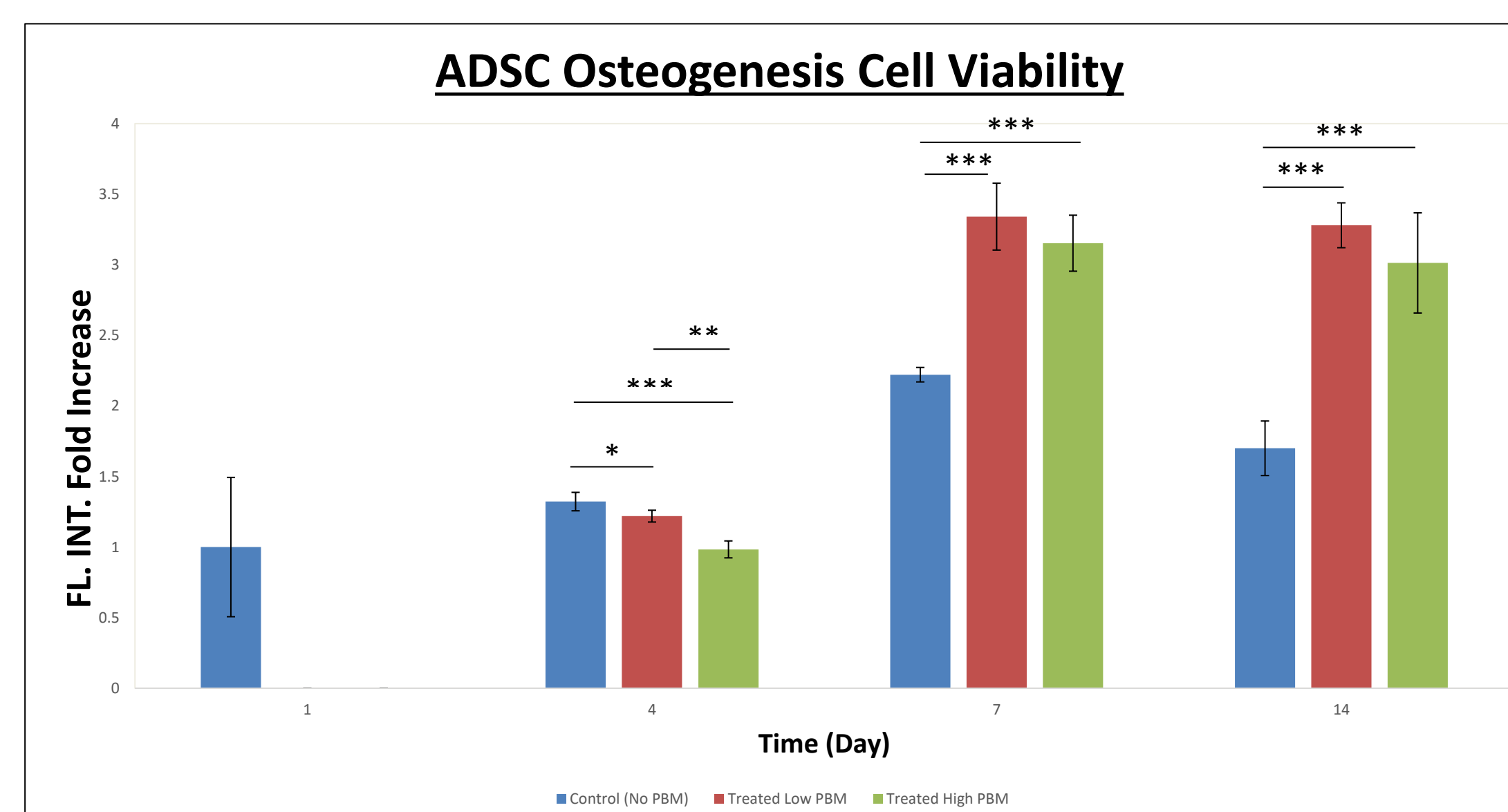


Figure 4. Cell viability of ADSCs under the same conditions was determined at days 4, 7, and 14. *p < 0.05 and *** p < 0.001.

Results Continued

Mitochondrial Morphology

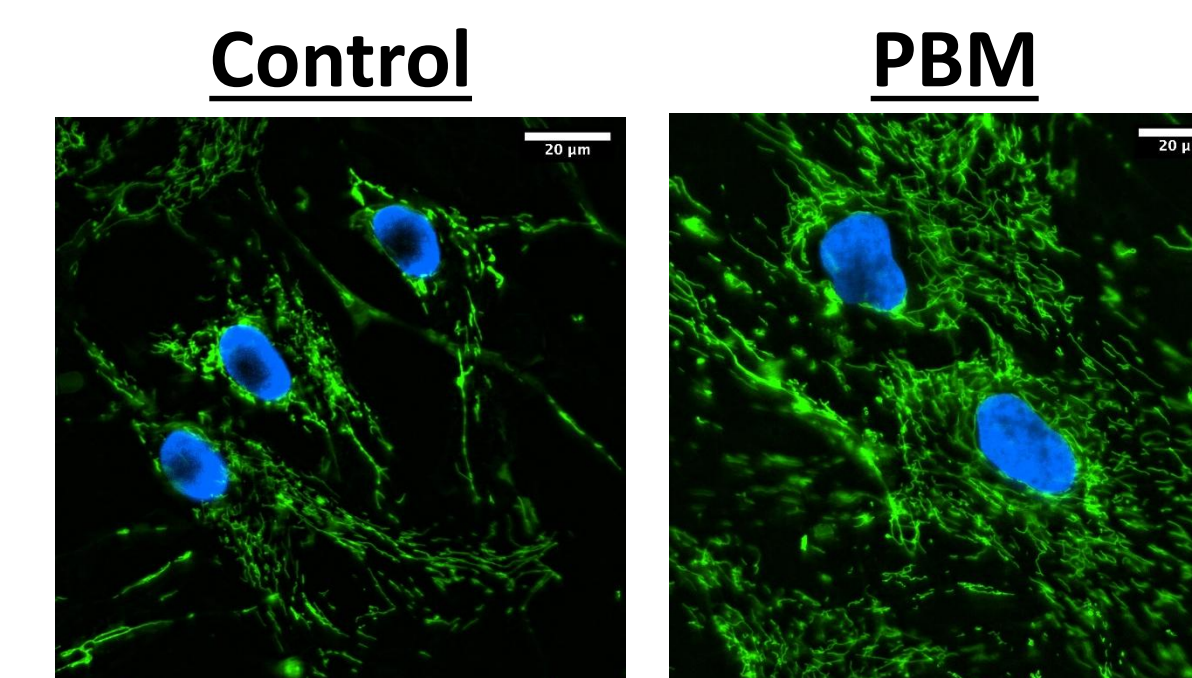


Figure 5. Mitochondrial Morphology of ADSCs in control and PBM treatments at Day 4.

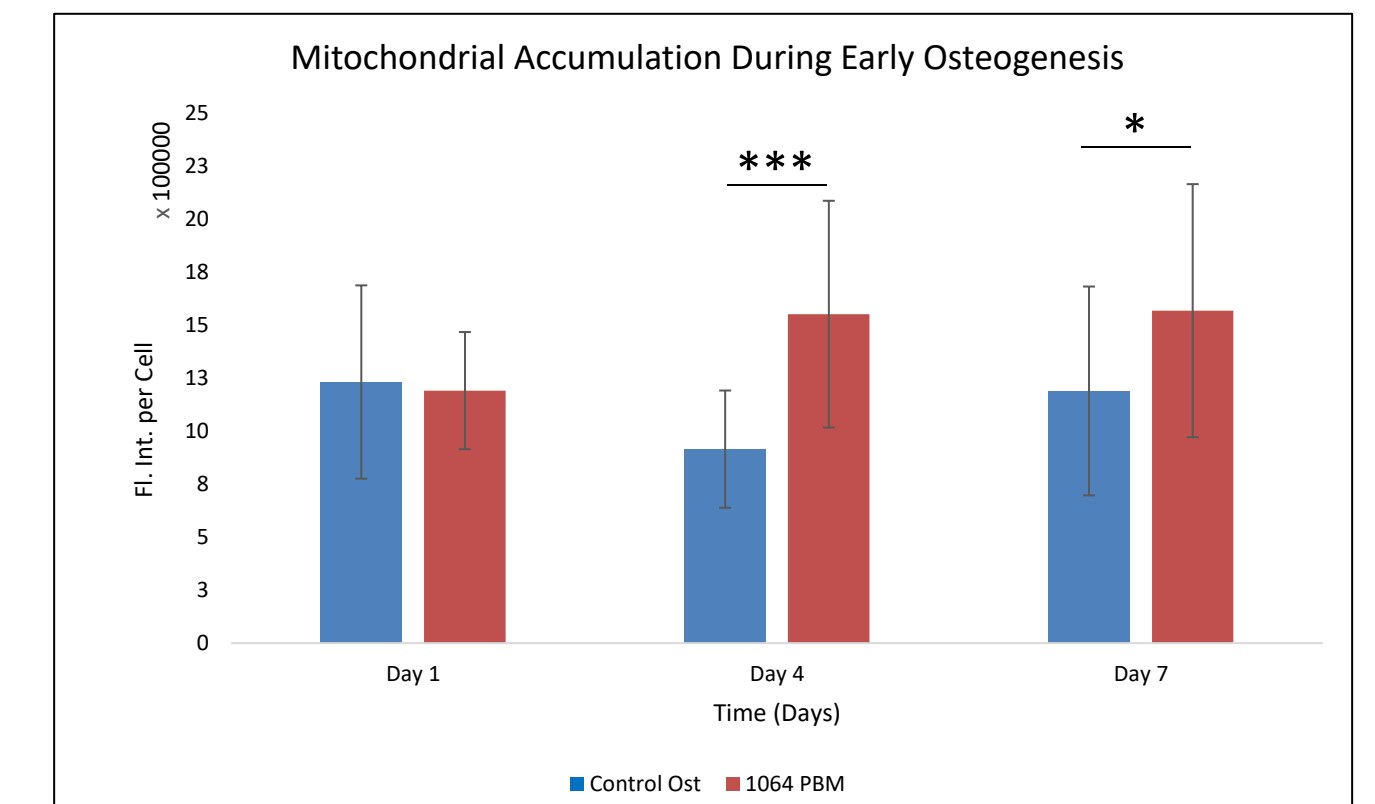


Figure 6. Quantification of mitochondrial accumulation during early osteogenesis at days 1, 4, and 7. * p<0.05 and *** p<0.001.

Discussion

Alizarin Red

- Significant differences on Days 14 and 21 between the control and both treatment groups were observed.
- No significant difference between the low and high PBM treatment groups.

MTT Assay

- Measures cell viability by determining the level of metabolic activity regulated by mitochondria.
- Significant difference in the cell viability on Days 4, 7, and 14 between the control and both treated groups.
- Significant difference on Day 4 between the low and high PBM treatment groups.

Mitochondrial Morphology

- Significant difference on days 4 and 7 between the control and PBM treatment.
- More mitochondria metabolic activity in PBM treatment compared to control.

Implications

- PBM is capable of enhancing the intended osteogenesis even though the cell type was intentionally mismatched, demonstrating improving bone health, particularly in patients with osteoporosis or fracture injuries, can be achieved using stem cells from various tissue types.
- Since MTT assay measures the cellular energy, the photonically enhanced stem cell differentiation is likely via mitochondrial remodeling and functionality.
- If directed to the bone marrow, PBM may be potentially used to mitigate the loss of bone mass associated with prolonged space travel.

References

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- McColloch, A., Liebman, C., Liu, H., & Cho, M. (2020). Altered Adipogenesis of Human Mesenchymal Stem Cells by Photobiomodulation Using 1064 nm Laser Light. *Lasers in Surgery and Medicine (Print)*, 53(2), 263–274. <https://doi.org/10.1002/lsm.23278>

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