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An in vitro bone model capable of continuous sensing and damage repair

Kalista Pattison

Team: Jeri Timlin, Kim Butler, Thomas Beechem, Kevin Strong, Stanley Chou

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Premise

- This project combines synthetic biology and materials principles to control the 3D architecture and remodeling of bone outside of a complete organism.
- Why bone?
  - Lightweight
  - Able to withstand high impact in a variety of dimensions and cyclic fatigue
  - Moderate amounts of stress lead to improved bone strength due to continuous sensing and damage repair, optimizing its chemical composition for increased mechanical performance.
- Impact: advance the field of self-healing materials, minimize animal research, and provide environmentally sustainable synthetic approaches to complex materials manufacturing.

Osteo -blasts, -clasts, and -cytes

Osteoblasts – bone producing cells, these arise from mesenchymal progenitor cells

Osteoclasts – bone resorbing cells, of the macrophage lineage

Osteocytes – terminally differentiated osteoblasts embedded in the matrix, able to sense mechanical load

Osteoblast and osteocyte differentiation
- Mesenchymal stem cell → Pre-osteoblast → Osteoblast → Osteocyte

Osteoclast differentiation
- Hematopoietic stem cell → Macrophage → Pre-osteoclast → Osteoclast

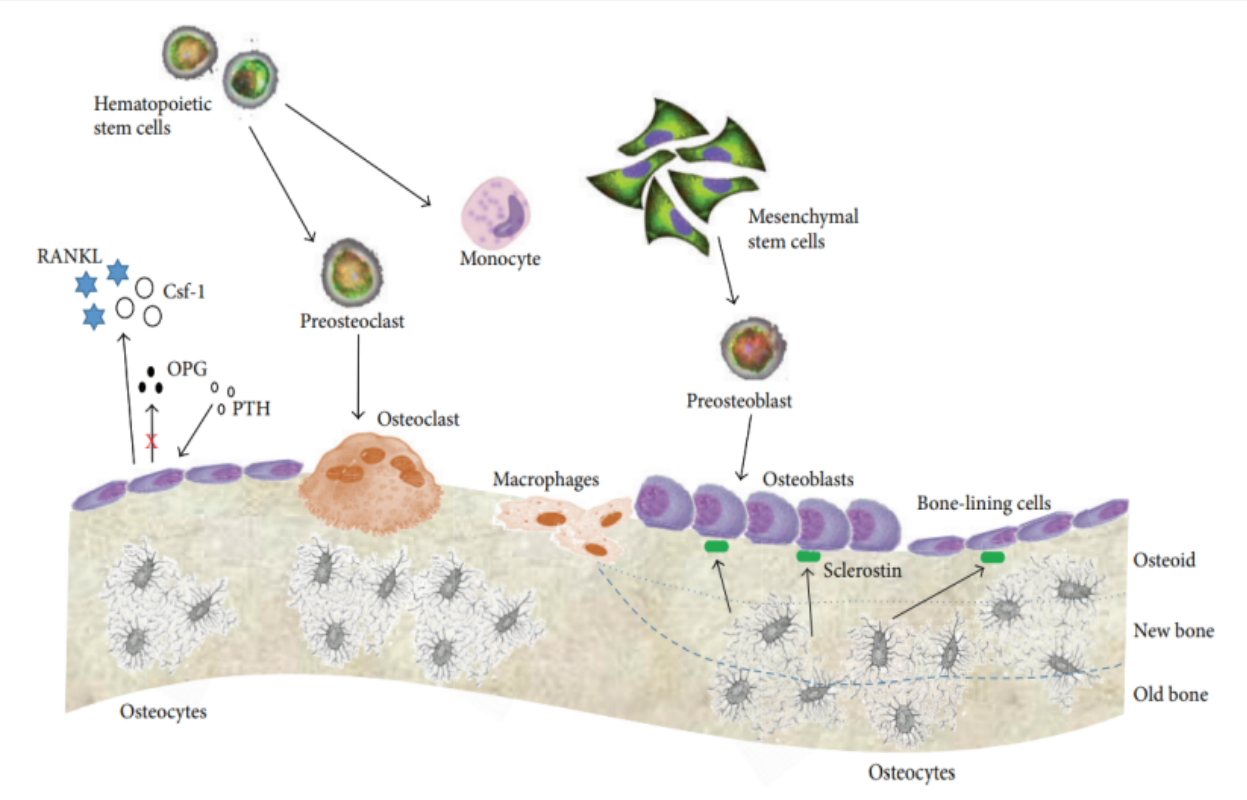
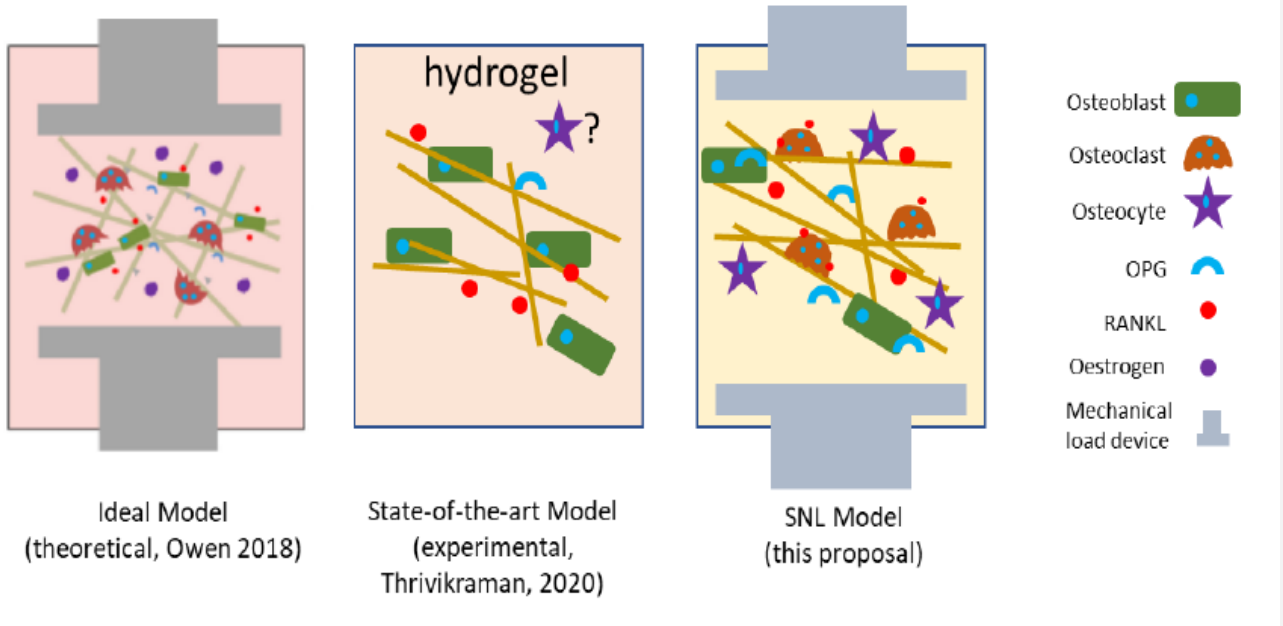


Figure 1. Pathways of differentiation for osteoclasts, osteoblasts, and osteocytes from hematopoietic stem cells and mesenchymal stem cells.

The Thrivikraman Model

We will leverage the Thrivikraman (2019) approach to rapid fabrication of vascularized and innervated cell-laden bone models with biomimetic intrafibrillar collagen mineralization to create a bone model that includes all three cell types and uses synthetic biology to produce key signaling molecules with spatial-temporal control.



1. Cell culture
maintain cells in culture (osteoclasts, osteoblasts)

2. Cell-laden hydrogels
type 1 collagen from rat tail tendon

3. Hydrogel mineralization
mineralization medium induces complete calcification of the collagen gels

SNL proposal

Confocal microscopy

Visualization of cell structures/proteins through staining and confocal microscopy.

- Matrix mineralization can be confirmed through alizarin red staining.
- Antibody staining to visualize osteocalcin, podoplanin, dentin matrix protein 1
- F-actin visualized with Alexa Fluor 488 phalloidin, and nuclei visualized with DAPI.

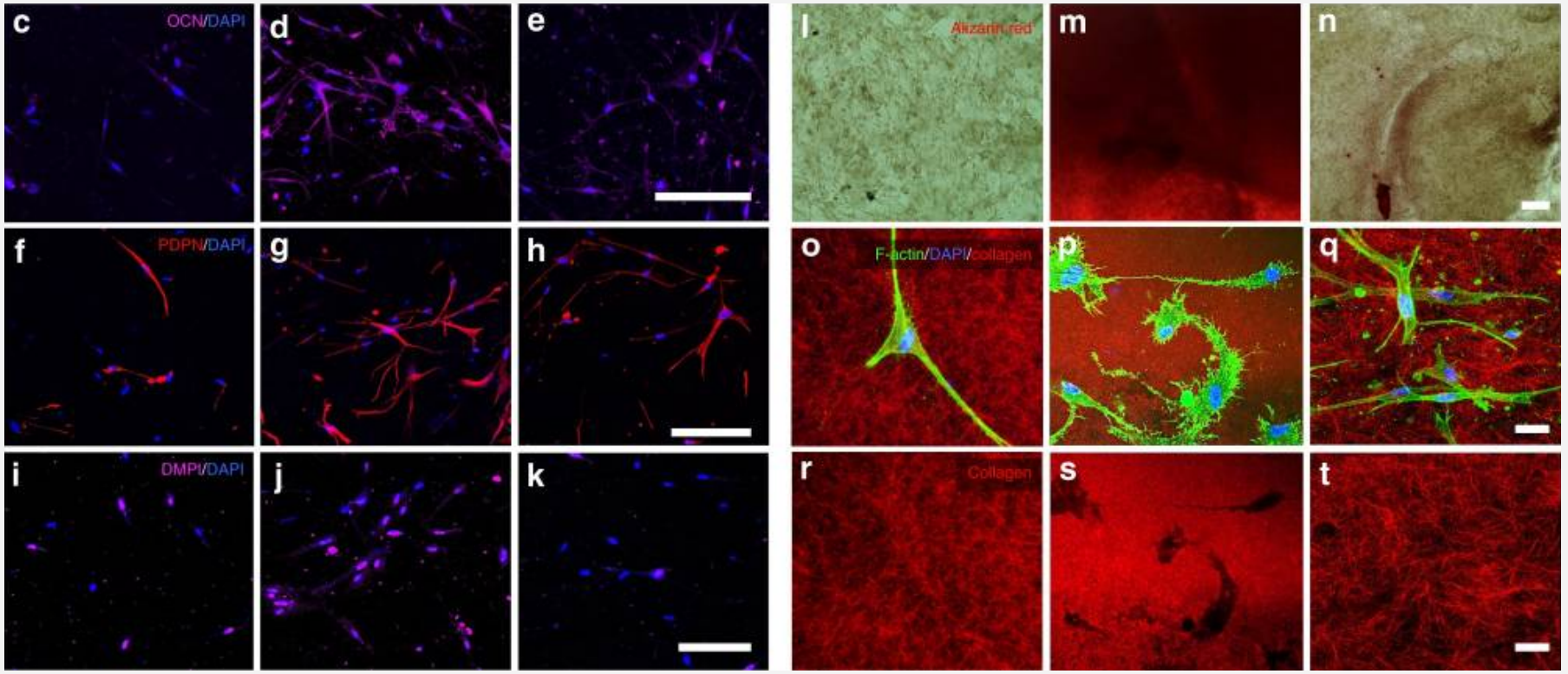


Figure 2. Cells labeled to visualize osteocalcin, podoplanin, dentin matrix protein 1, mineralization, nuclei, cytoskeleton, and lacunae-like regions within the matrix where cells with reside.

Osteoclast differentiation

RANKL and M-CSF

Osteoclasts differentiate from macrophages with the help of: macrophage colony-stimulating factor (*M-CSF*) and receptor activator of nuclear factor kappa-B ligand (*RANKL*). These differentiated cells are positive for tartrate-resistant acidic phosphatase (*TRAP*), an enzyme with roles in skeletal development, collagen synthesis and degradation, bone mineralization, degrading osteopontin, etc.

OPG/RANKL ratio

OPG (osteoprotegerin) is also secreted by osteoblasts and is a decoy receptor for RANKL. Protective against bone loss.

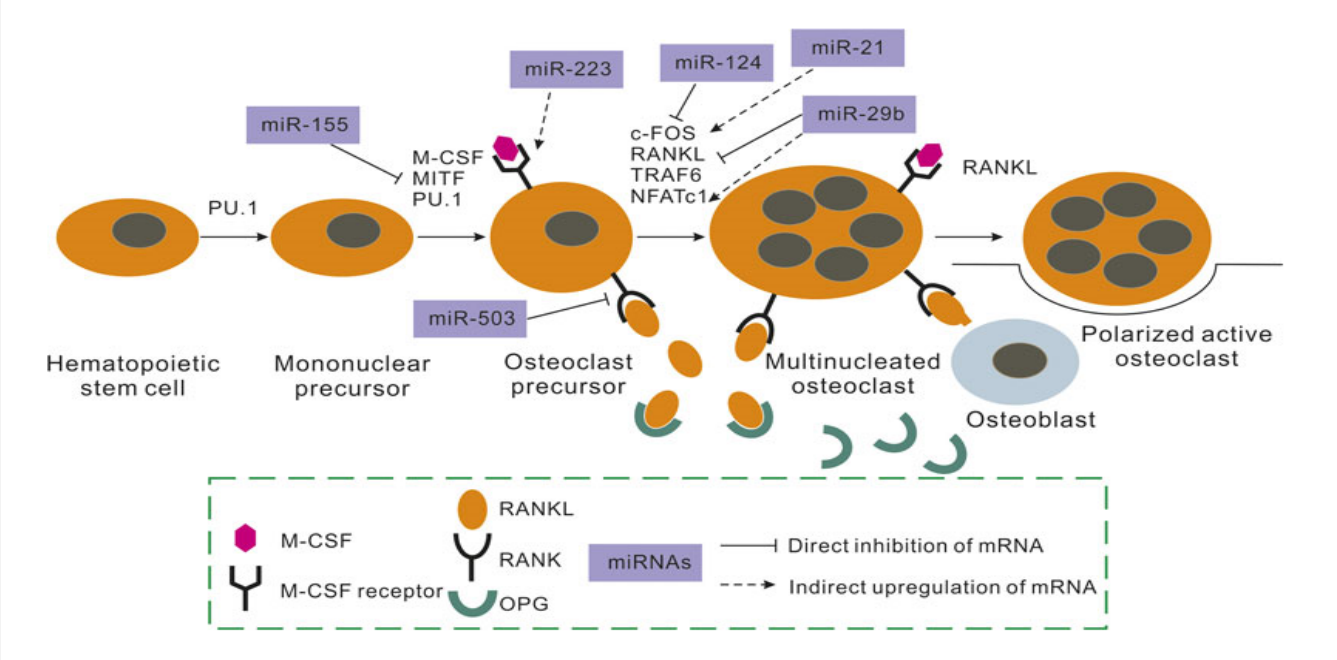


Figure 3. Osteoclast differentiation via receptor activator of nuclear kappa-B ligand (RANKL) and osteoprotegerin (OPG).

PART 1: RAW 264.7 to Osteoclasts

We need to create our own osteoclasts from macrophages/monocytes.

How?

- Prepare an osteoclast differentiation media containing RANKL
- Plate cells in media and observe morphology until large multinucleated osteoclasts appear (3-6 days)
- Separate osteoclasts by nuclei count using a serum gradient where the osteoclasts with the most nuclei will sink to the bottom.

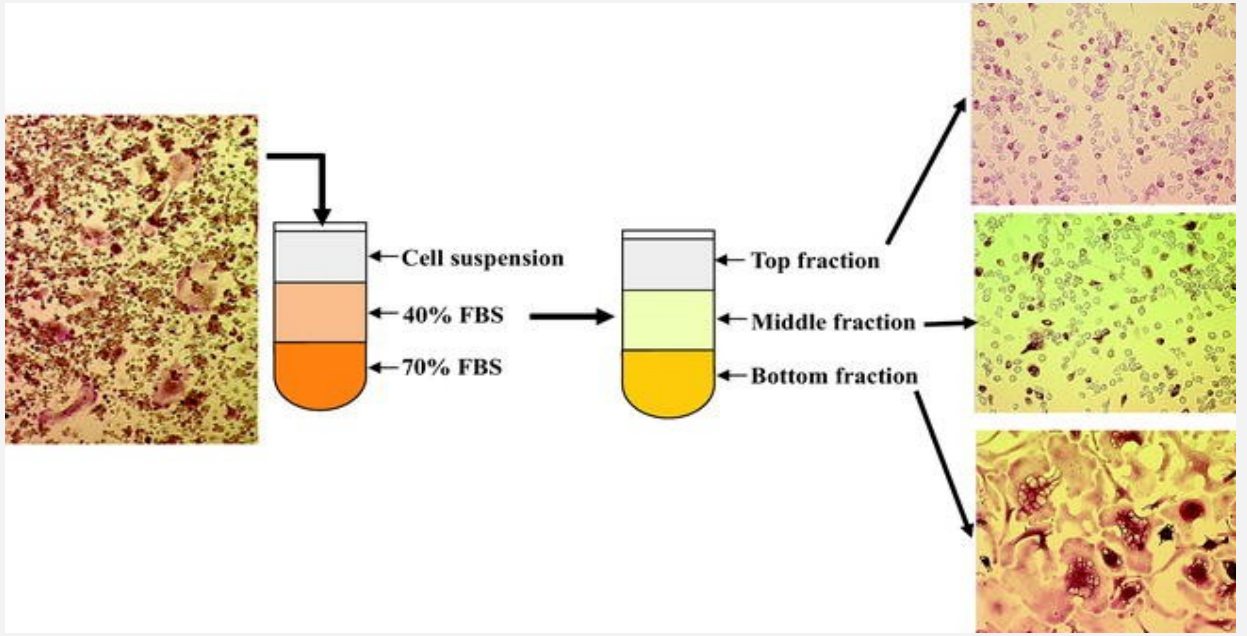
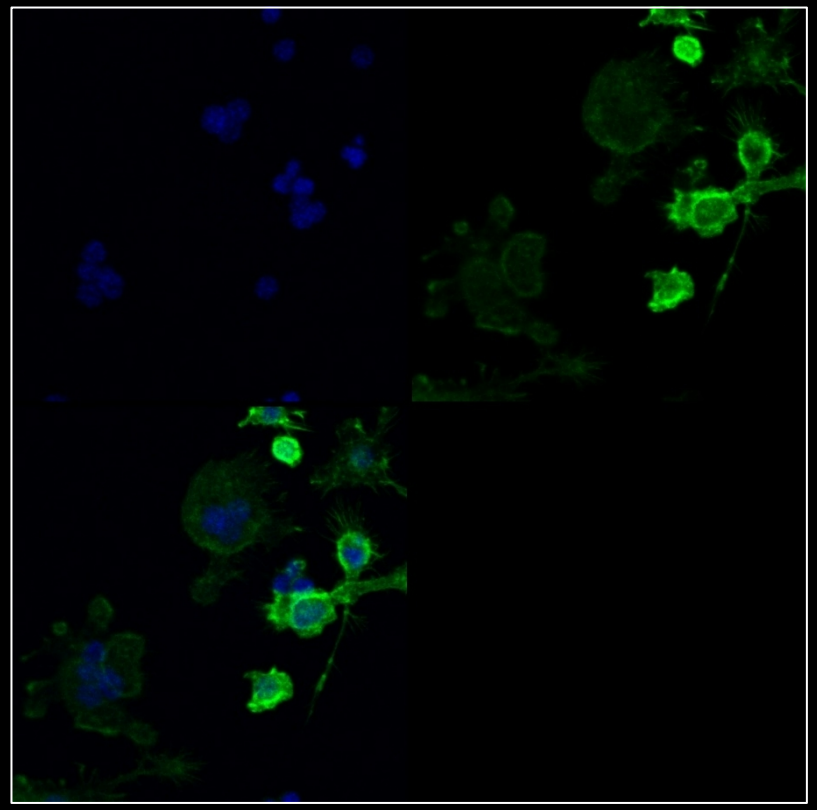
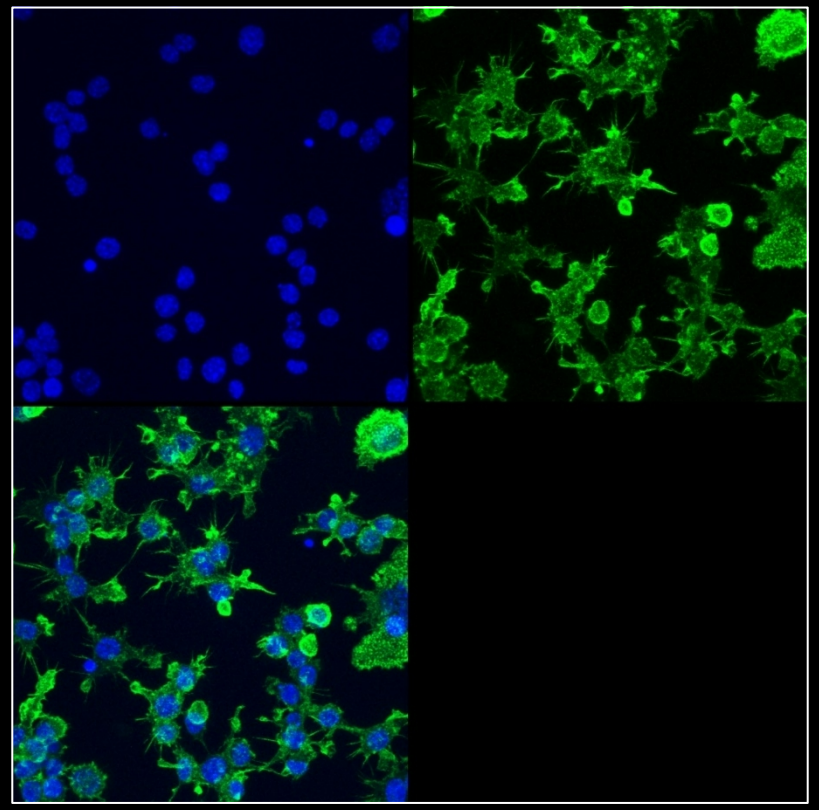


Figure 4. Osteoclast purification via serum gradient.

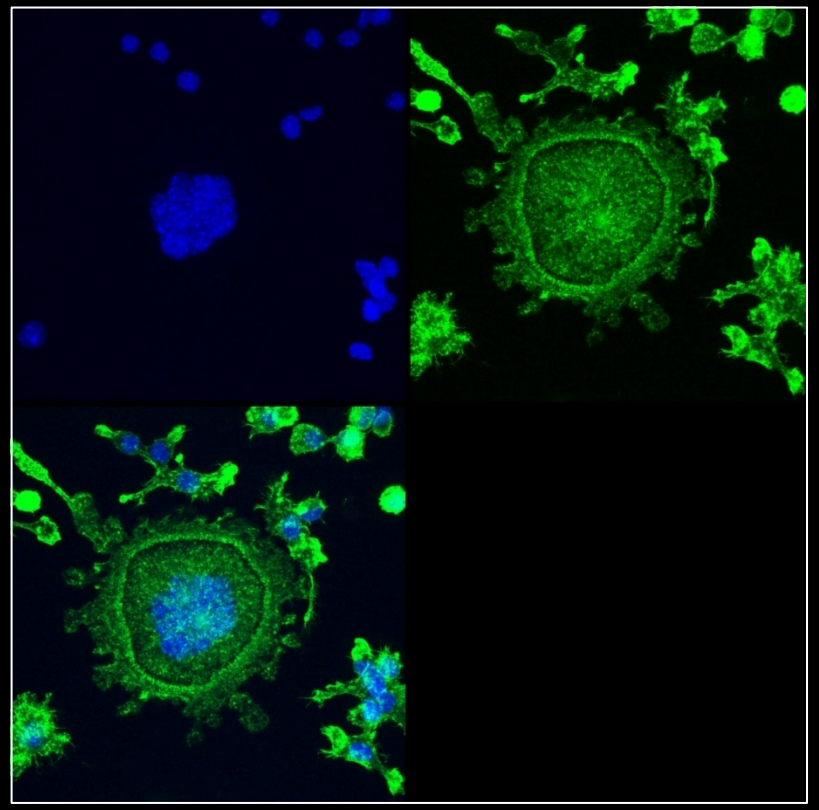
Osteoclast Imaging



20210527 Middle Layer

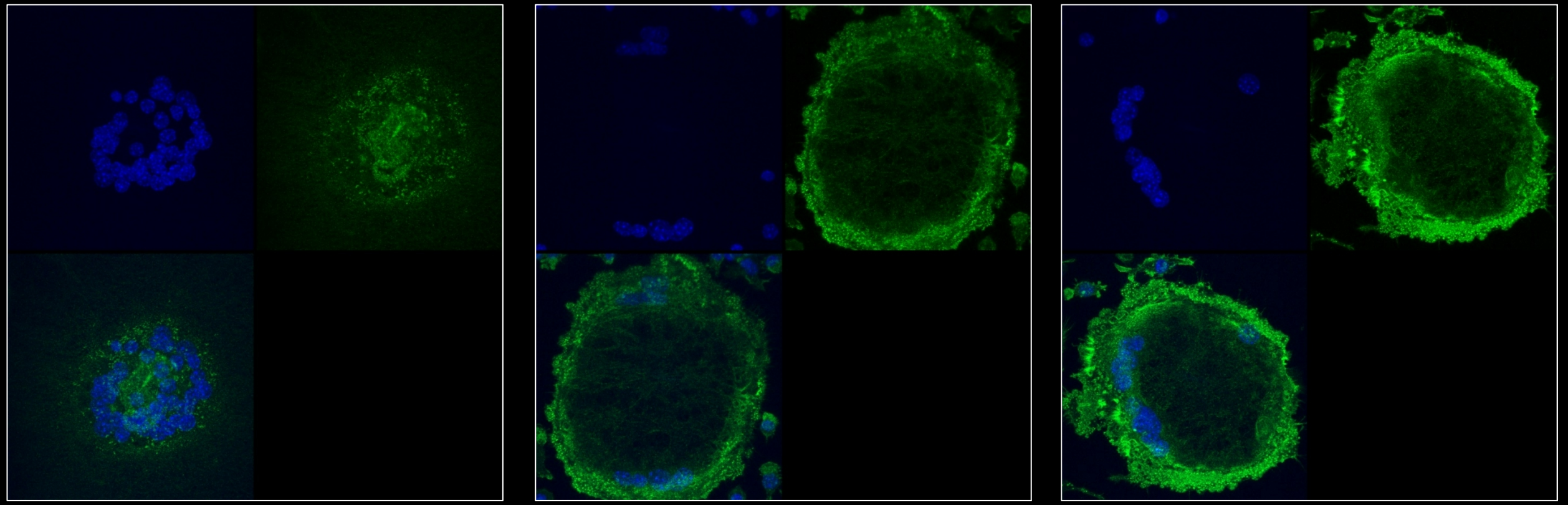


20210608 Bottom Layer (RAWS)



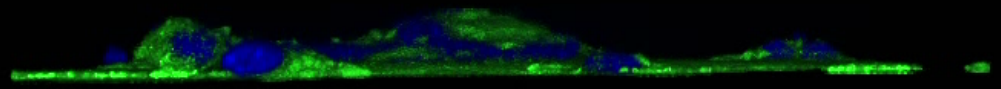
20210608 Bottom Layer (OC)

Osteoclast Imaging



20210608 Bottom Osteoclasts (all images)

3D Osteoclast



Verify osteoclast production

Next step is to verify osteoclast production through: pit formation assays and immunofluorescent staining are among some methods.

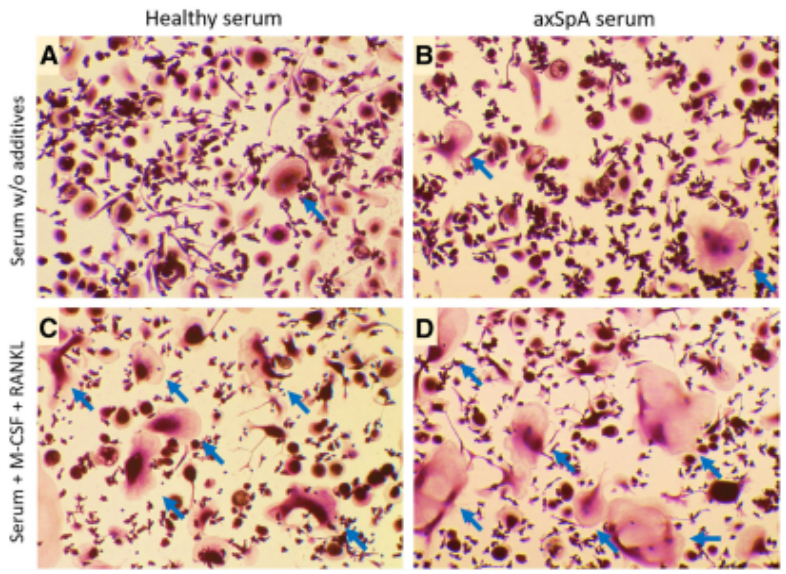


Figure 5. TRAP stained cells to demonstrate osteoclast creation due to MCSF and RANKL added to serum containing monocytes/macrophages.

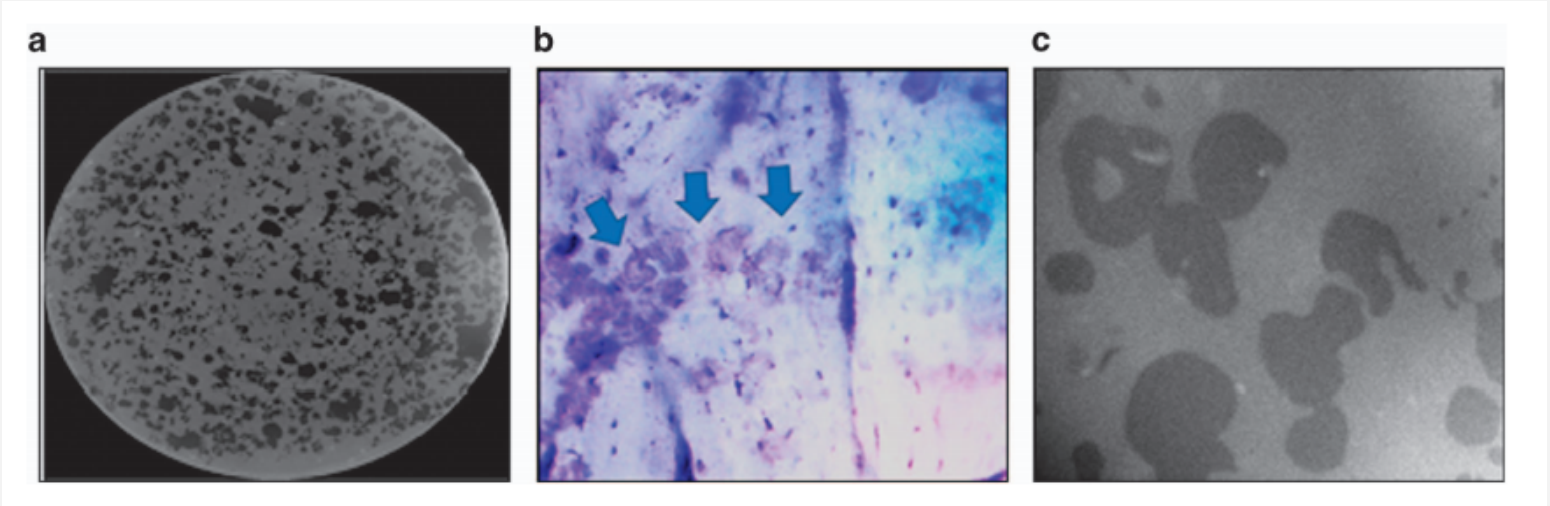


Figure 6. A: dentin slices with resorption pits visible following osteoclast culturing. B: bone slices with resorption pits following osteoclast culture, collagen exposed through resorption are stained blue. C: osteo assay surface multiple-well plates after osteoclast culturing.

Citations

FIGURE 1: Qin YX, Hu M (2014) *Mechanotransduction in musculoskel-etal tissue regeneration: effects of fluid flow, loading, and cellular-molecular pathways* . Biomed Res Int. DOI:10.1155/2014/86342 1.

FIGURE 2: Thrivikraman G, Athirasala A, Gordon R, Zhang L, Bergan R, Keene DR, et al. (2019) *Rapid fabrication of vascularized and innervated cell-laden bone models with biomimetic intrafibrillar collagen mineralization*. Nature Communications. 10:3520. DOI: 10.1038/s41467-019-11455-8.

FIGURE 3: Jing, Dian & Hao, Jin & Shen, Yu & Tang, Ge & Li, Mei-Le & Huang, Shi-Hu & Zhao, Zhi-He. (2015). *The role of microRNAs in bone remodeling*. International journal of oral science. 7. 10.1038/ijos.2015.22.

FIGURE 4: Collin-Osdoby P., Osdoby P. (2012) *RANKL-Mediated Osteoclast Formation from Murine RAW 264.7 cells*. In: Helfrich M., Ralston S. (eds) Bone Research Protocols. Methods in Molecular Biology (Methods and Protocols), vol 816. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-61779-415-5_13

FIGURE 5: Korkosz M, et al. (2018) *Sera of patients with axial spondyloarthritis (axSpA) enhance osteoclastogenic potential of monocytes isolated from healthy individuals.* BMC Musculoskeletal Disorders. 19:434. DOI: https://doi.org/10.1186/s12891-018-2356-4

FIGURE 6: Marino S, Logan JG, Mellis D, Capulli M. *Generation and culture of osteoclasts*. *Bonekey Rep*. 2014;3:570. Published 2014 Sep 10. doi:10.1038/bonekey.2014.65

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Shafieyan Y, Tiedemann K, Goulet A, Komarova S, Quinn TM. 2012. *Monocyte proliferation and differentiation to osteoclasts is affected by density of collagen covalently bound to a poly(dimethyl siloxane) culture surface.* J Biomed Mater Res Part A 2012:100A:1573–1581.