MASSACHUSETTS GENERAL HOSPITAL
HARVARD MEDICAL SCHOOL

Center for Skeletal Research
MGH Endocrine Unit

Bone Cells Core

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Bone Marrow Cultures

Flush bone marrow from femur/ tibia (cut both end and flush the BM with 1 ml of α-MEM ice cold)

- Collect cells and count cells (take 100-200µl of cell suspension, add equal volume of 2% acetic acid to lyse red blood cells. Let sit for 10 min @ room temp, count- remember that cells were diluted 1:2 !) and resuspend the cells in αMEM 10%FBS 1%PS

FOR Osteoclasts formation

- Resuspend the cells to a final density of $1.5x10^6$ cells/ml Plate cells at $1.5x\ 10^6$ cells/ml in 24-well plates (0.5 ml/well). Use only the 8 central wells and add PBS or H2O to the outside wells. Cells are plated in α-MEM+10% FBS+1% PS and Dexamethasone 100nM (protocol from Takahasi et al. Endocrinology 1988)
- Add treatment : vit D (10nM) or PTH (10nM) to induce OCs differentiation
- Every other day change ½ volume (250 ul) and replace with fresh medium containing 2X Dexamethasone and treatment
- Ocs will form within 2 weeks

FOR CFU-s experiment

- Resuspend the cells to a final density of $1x10^6$ cells/ml and plate into a 6-well plate (2ml/well), Add mineralization medium (50µg/ml of L-Ascorbic Acid and 10mM β-glycerophosphate) for CFU-osteoblasts
- Change medium 3 times/week
- Stain for ALK Phopsh and count colonies bigger than 2 mm (Cfu-Ob)
- Stain with crystal violet for total cells count (CFU-f)