Center for Musculoskeletal Research
Massachusetts General Hospital
50 Blossom Street, Thier 10 & 11
Boston, MA 02114-2696

Email: CSRMAIL@PARTNERS.ORG
Website: www.csr-mgh.org

Musculoskeletal Cell Core
Directors: Paola Divieti Pajevic, MD, PhD
Lou Gerstenfeld, PhD

Email: BoneCell@bu.edu

Bone Marrow Cells Isolation

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 100-200 µl 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 µl) and replace with fresh medium containing 2X Dexamethasone and treatment
- OCs will form within 2 weeks
- For OCs visualization perform TRAP staining

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 B-glycerophosphate) to induce CFU-osteoblasts (CFU-Ob)
- Prepare 1000X stock of both AA and BGPO and store them at -20C protected from light. Add them fresh every time (add to αMEM complete medium and filter
- 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)

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ALK Phosphatase staining:

Reagents needed:

- 3.7% paraformaldehyde (PFA) or 10% buffered Formalin
- ALP staining solution:
  - Naphtol AS-MX phosphate (Sigma N-4875) 5mg
  - NN-Dymethylformamide 0.25 ml
  - 0.1M Tris buffer (pH 8.5) 50ml
  - Fast Blue BB salt (Sigma F-0250) 30 mg
- First dissolve the Naphtol AS-MX in the NN Dymethylformamide, then add Tris and the add the fast blue salt. Dissolve completely and filter (0.45um to remove particle)
- Protect from light and keep on ice

Procedure:

- Aspirate medium
- wash the plated 3 times with PBS (2ml/well)
- fix with PFA or Formalin at RT for 30 min
- wash 3 times with PBS
- Incubate for 30 min at RT with the ALK SOLUTION (protect from light)
- Wash the cells 3 times with PBS or DW
- Count the colonies

NB: you need to count ALK colonies (CFU-Ob prior to proceed with the CV since it will stain over the ALK)

Crystal Violet (CV) staining of cells and Clone counting (from Timothy lane)

Reagents:

- Stocks
  - 3.7% paraformaldehyde (PFA) or 10% buffered Formalin
  - 0.05% Crystal violet dilute in distilled water and filtered (0.45 uM before use)

Procedure: (skip the fixation if done it above)

- Aspirate medium
- wash the plated 3 times with PBS (2ml/well)
- fix with PFA or Formalin at RT for 30 min
- wash 3 times with PBS
- STAIN for 30 mi with 0.05% CV
- Wash 2-3 times with tap water
- Dry and count colonies/take images

Reading CV staining (OD450)

- Add 0.1-1 ml of methanol to the plate to solubilize the dye
- Read the plate directly or by taking an aliquot of the staining (OD 450)
- NB: The cells do not lyse and the morphology is well preserved

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