Bone Marrow Macrophages

Material

- Fycoll Paque plus (GE cat #71-7167-00 AG)
- 10 cm Dishes
- 4-6 weeks old mouse
- 70% ethanol
- RANKL (R&D or Shenandoah. Prepare stock at 0.1 mg/ml)
- Mouse M-CSF (R&D or Shenandoah. Prepare stock at 0.1 mg/ml)
- Surgical instruments (small scissors n=2; small forceps n=2, scalpel)
- Bead sterilizer
- Alpha MEM +10%FBS +1 %AA (complete medium)
- 5 and 10mL syringes
- 40 uM cell strainer

Procedure:

Day 1:
For isolation of mouse BMMs from WT mice, femurs and tibias of 4–6-week-old mice are aseptically removed and BMSCs are flushed out the marrow cavity with α-MEM (no serum added) or sterile PBS (usually 5 ml for each bone).
Cells are collected in a 50ml tube, centrifuged at 1000 RPM for 5-10 min and supernatant is removed and pellet is gently resuspended in 10 ml of α-MEM containing 10% FBS 1% AA and cultured in 10 cm dishes at 37 °C and 5% CO₂ for 24-48 hr.

Day 2:
Gently remove non-adherent cells with a 10 ml pipette and transfer to a new 50 ml conical tube. Wash gently the dish one more time with 10ml of α-MEM containing 10% FBS 1% AA and add to the same 50-ml tube Centrifuge at 1000RPM for 10 min
Suspend the cells in 5 ml of complete medium (α-MEM containing 10% FBS 1% AA). Use another 5 ml of medium to rinse the tube.
In a new 50ml conical tube add 15 ml of Fycol hypaque (Farmacia) and gently layer the 10ml of cell suspension

MGH CSR Bone Cells Core Protocols http://www.csr-mgh.org/protocols/
Spin cells at 1500 rpm for 15 min
Pull off the first 10ml of medium off the top and collect the interface with a 10 ml pipette into a 50 ml conical tube
Bring up the volume to 50 ml with complete medium.
Centrifuge for 10 min at 1000RPM and resuspend the cells in α-MEM containing 10% FBS 1% AA. 2 ml/mouse.
Cells are now ready for downstream application.
For osteoclastogenesis:
Count the cells and plate at 20,000 cells/well in 96 well plate or 50,000 cells/well in 12 well plate and treat with MCSF for 48-72 hr (MCSF 20-50 ng/ml)
After 48-72 hr start RANKL treatment (50 ng/ml), in addition to MCSF treatment. Replace medium every 3 days
Osteoclasts usually form after 2-3 days in RANKL TC setting