Isolation of bone marrow macrophage

Macrophage medium
Base: DMEM
20% L-cell medium supplement (in -80C freezer)
1% Antibiotics (5mL aliquot in -20C)
1% Glutamine (Gibco#25030-100x; 5mL aliquot in -20C)
10% Heat inactivated FBS (50mL aliquot in -20C)

Procedures:

1. Prepare a 10mL syringe/23G needle filled with DMEM

2. Trim the femur to get rid of the muscle, and cut a tiny piece of bone at the 2 ends of the femur so to have an inlet and outlet of the medullary cavity

3. Inject DMEM through the little hole (opening of the medullary cavity) to rinse out the bone marrow

4. Collect the bone marrow and pipette up and down to break down the clump

5. Filter the cell suspension with 40um cell strainer, spin at 2000 rpm for 5 min

6. Resuspend the cell pellet with 10mL macrophage medium and plate in a 100mm plate (petri dish)

7. Incubate for 5-6 days in macrophage medium for full differentiation and then change medium every day for 2-3 more days

8. Replate cells for experiments (using cell dissociation buffer for detachment)

1Femur = 2 of 100mm plates