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Immune Cell Isolation from Mouse Femur Bone Marrow

Xiaoyu Liu [1, 2] and Ning Quan [1, 2*]

[1]Division of Biosciences, The Ohio State University, Columbus, USA; [2]Institute for Behavioral Medicine Research, The Ohio State University Wexner Medical Center, , Columbus, USA
[*]For correspondence: quan.14@osu.edu

[Abstract] The bone marrow is the site of hematopoiesis and contains mixed population of blood cells including erythrocytes, granulocytes, monocytes, dendritic cells, lymphocytes and hematopoietic stem cells. The following protocol provides a simple and fast method for isolation of bone marrow immune cells (no erythrocytes) from mouse femurs with a yield of approximate 8 x 10^7 cells in 5 ml culture media (1.6 x 10^4 cells/µl). Further isolation or flow cytometric analysis might be required for study of specific immune cell types.

Materials and Reagents

1. Sterile paper towel
2. Sterile surgical pad (Direct Resource, catalog number: 19015742)
3. 23-gauge (or 25-/26-gauge) needle (BD Biosciences, catalog number: 305145)
4. 10 ml syringe (BD Biosciences, catalog number: 309604)
5. 70 µm nylon cell strainer (Falcon, catalog number: 352350)
   Note: Currently, it is "Corning, Falcon®, catalog number: 11995-065".
6. 50 ml conical tube (Falcon, catalog number: 21008-940)
   Note: Currently, it is "Corning, Falcon®, catalog number: 21008-940".
7. 5 ml syringe plunger (BD Biosciences, catalog number: 309646)
8. Adult mice (> 6 weeks, any strain) (*e.g.,* C57BL/6)
9. Hank's balanced salt solution (HBSS), no Calcium, no Magnesium, no phenol red (Life Technologies, catalog number: 14175095)
   Note: Currently, it is "Thermo Fisher Scientific, Gibco™, catalog number: 14175095".
10. DMEM medium, high glucose, pyruvate, L-glutamine (Life Technologies, catalog number: 11995-065)
    Note: Currently, it is "Thermo Fisher Scientific, Gibco™, catalog number: 11995-065".
11. 70% ethanol
12. Fetal bovine serum heat inactivated (FBS) (Sigma-Aldrich, catalog number: F9665)
13. Ammonium chloride (NH_4Cl) (Sigma-Aldrich, catalog number: 213330)
14. Potassium bicarbonate ($KHCO_3$) (Sigma-Aldrich, catalog number: 237205)
15. Disodium edetate (Sigma-Aldrich, catalog number: D2900000)
16. RBC lysis buffer (see Recipes)
17. DMEM medium (see Recipes)

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Equipment

1. Blunt-end sterile scissors (Thermo Fisher Scientific, Fisher Scientific, catalog number: 08-950)
2. Sharp sterile scissors (Thermo Fisher Scientific, Fisher Scientific, catalog number: 08-940)
3. Sterile forceps (Thermo Fisher Scientific, Fisher Scientific, catalog number: 08-890)
4. Hausser™ Levy™ Hemacytometer Chamber Set (Thermo Fisher Scientific, Fisher Scientific, catalog number: 02-671-55A) or coulter Z2 cell and particle counter (Beckman Coulter, catalog number: 383550)
5. Refrigerated centrifuge
6. Sterile culture hood
7. CO_2 rodent euthanasia chamber

Procedure

1. Euthanize the mouse with CO_2 and place mouse onto a sterile surgical pad in a sterile hood. Sterilize the mouse abdomen area and skin of hindlimbs with 70% ethanol (Figure 1).

Figure 1. Sterilization of mouse abdomen area and skin of hindlimbs

2. Open the abdominal cavity with blunt-end sterile scissors and remove the surface muscles and find the pelvic-hip joint (Figure 2).

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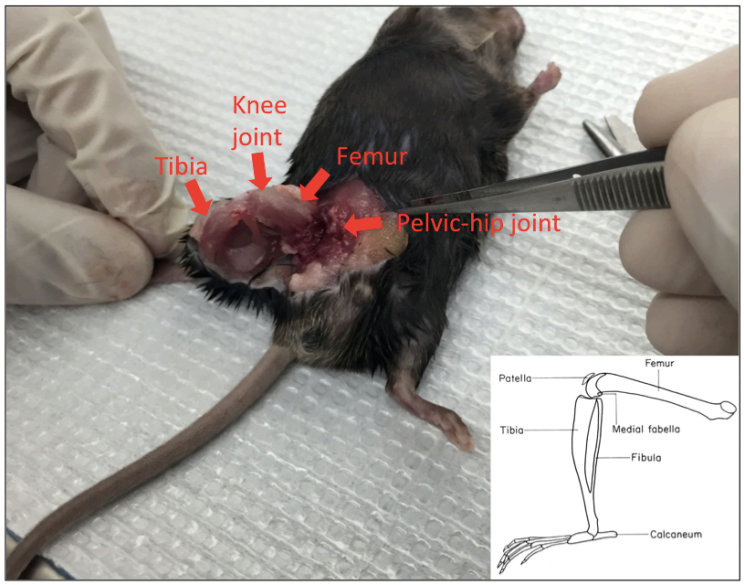

Figure 2. Find the pelvic-hip joint. Bone anatomy reference: http://www.informatics.jax.org/cookbook/figures/figure41.shtml

3. Cut off the hind leg above the pelvic-hip joint with sharp sterile scissors (Figure 3). Cut off the tibia from the hind leg below the knee joint with sharp sterile scissors (Figure 4).

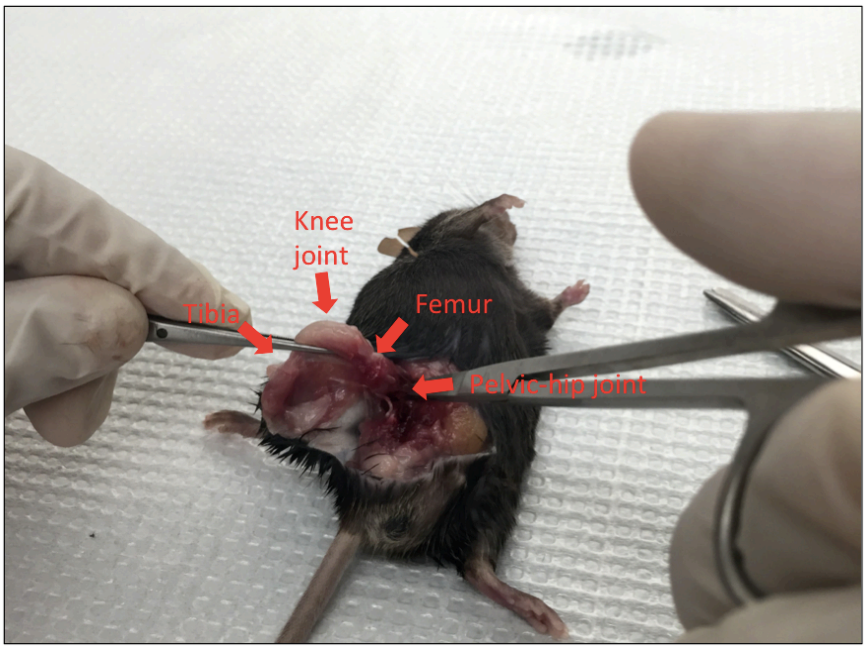

Figure 3. Cut off the hind leg above the pelvic-hip joint

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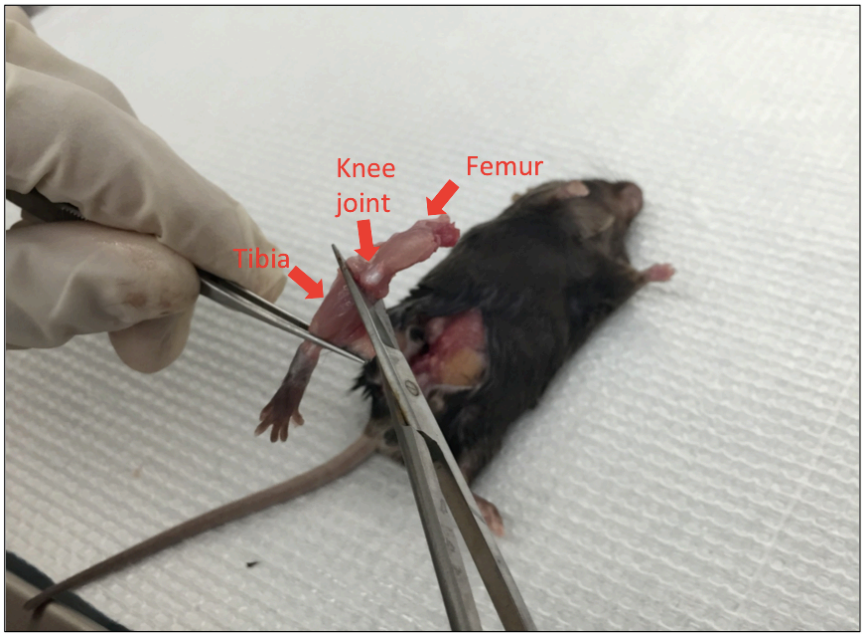

Figure 4. Cut off the tibia at knee joint

4. (Optional) If higher yield of bone marrow cells is needed, tibia can also be used for bone marrow cell isolation. Cut at the tibia ankle joint to dissect the tibia. The following procedures can be applied to both femur and tibia.
5. Remove the muscles and residue tissues surrounding the femur with sterile forceps and scissors (Figure 5).

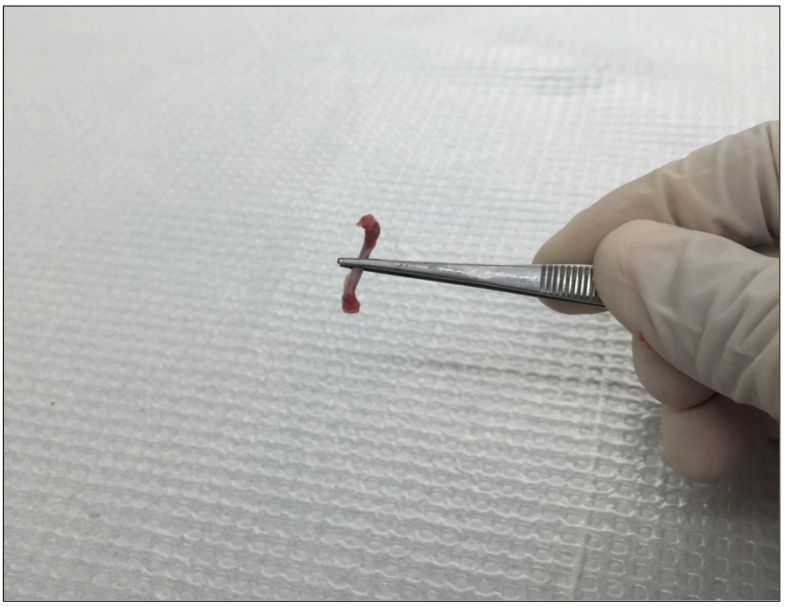

Figure 5. Remove the muscles and residue tissues surrounding the femur

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6. Cut the femurs at both ends with sharp sterile scissors (Figure 6). Use a 23-gauge (some literature suggests 25-or 26-gauge) needle and a 10 cc syringe filled with ice-cold HBSS to flush the bone marrow out onto a 70 µm nylon cell strainer placed in a 50 ml Falcon conical tube (Figure 7). Use all the 10 ml HBSS or until the flow through turns white.

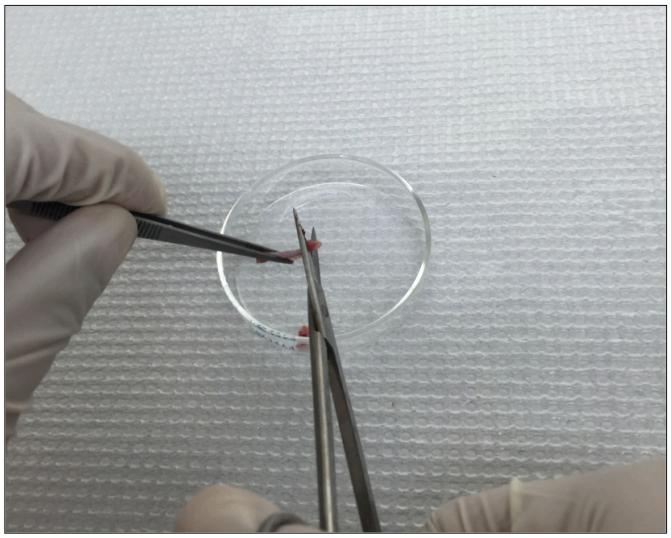

Figure 6. Cut femurs at both ends

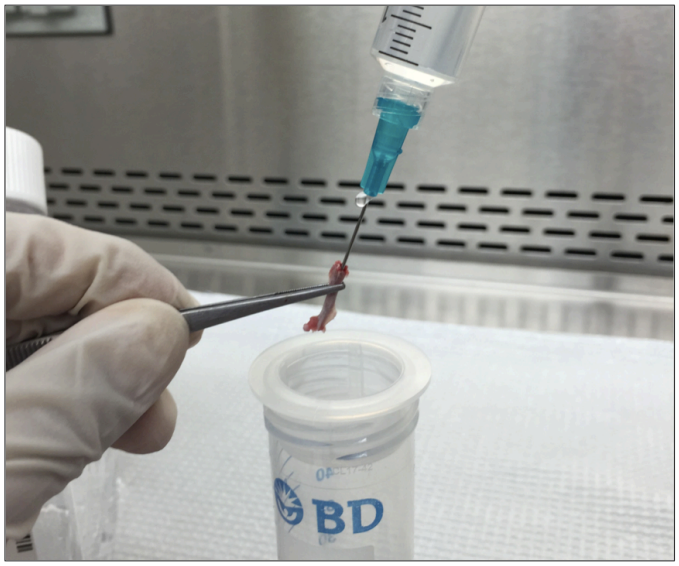

Figure 7. Flush the bone marrow onto the cell strainer with HBSS

7. (Optional) In case some residue bone marrow cells could not be flushed off (very few bone marrow visible in the flow through or the yield is significantly less, *e.g.,* < 1 x 10^7 cells), scrape the inner surface of the femur with the needle and flush with extra ~5 ml HBSS (Figure 8).

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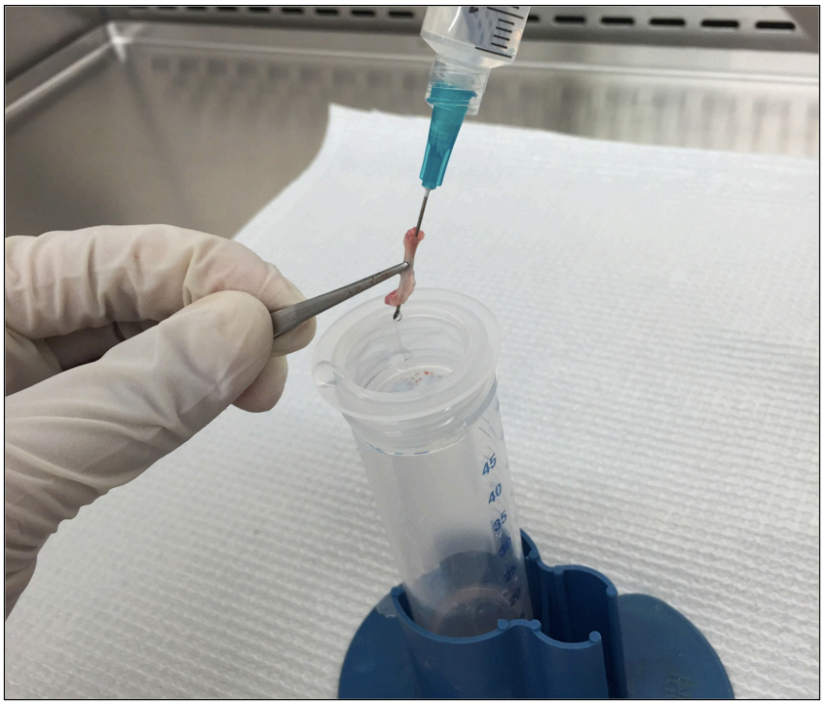

Figure 8. (Optional) Scrape the inner surface of femur with needle

8. Smash the bone marrow through the cell strainer with a 5 ml plunger (Figure 9). Wash the strainer with another ~5 ml HBSS.

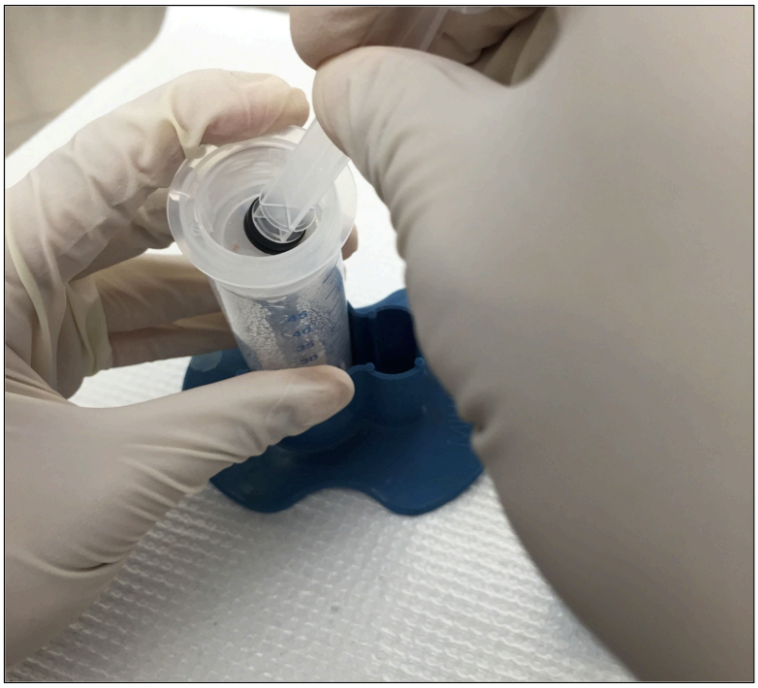

Figure 9. Smash the bone marrow cells through the cell strainer with a 5 ml plunger

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9. Centrifuge cells at 1,500 rpm for 7 min at 4 °C. Discard the supernatant and blot on paper towel (Figure 10).

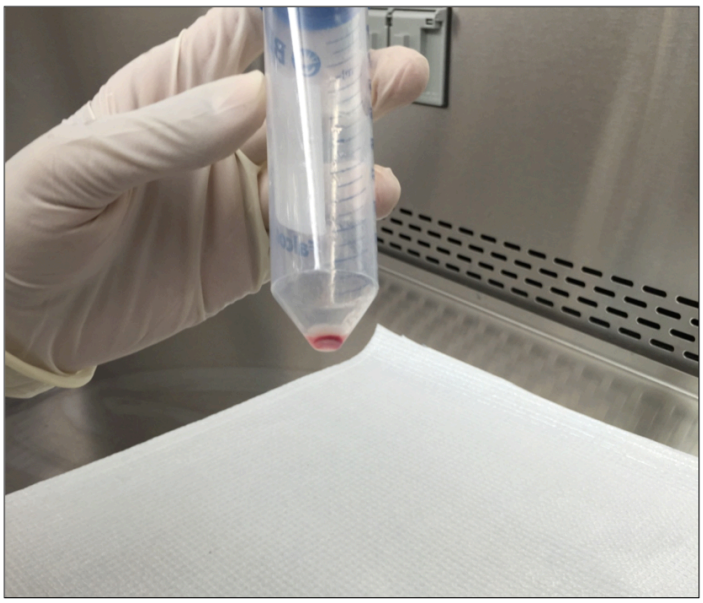

Figure 10. Cell pellet before RBC buffer resuspension

10. Resuspend the cell pellet with 1 ml RBC lysis buffer (for each mouse). Incubate for 5 min at room temperature or 2 min at 37 °C, and neutralize the lysis buffer by adding 5 ml FBS.
11. Centrifuge cells at 1,500 rpm for 7 min at 4 °C. Discard the supernatant and blot on paper towel. Resuspend the cell pellet with appropriate media for the next step of assay such as 5 ml DMEM medium containing 10% FBS. Cells are then placed on ice.
12. Count the bone marrow cells with a hemocytometer or a Beckman Z2 coulter counter. Cells are ready for assays or culture. Cells can stay viable on ice for at least 5 h. It is recommended to perform the experiment (culture or assays) right after isolation for best results.

Recipes

1. RBC lysis buffer
   0.16 M NH_4Cl, 10 mM $KHCO_3$, and 0.13 mM EDTA, dissolved in sterile H_2O and stored at 4 °C
   For 500 ml, 4.28 g NH_4Cl, 0.5 g $KHCO_3$, 0.024 g Disodium EDTA
   It is recommended to prepare fresh RBC lysis buffer for the experiment. RBC lysis buffer will be stable at 4 °C for at least 1 month.
2. DMEM medium
   DMEM medium, high glucose, pyruvate, L-glutamine supplemented with 10% FBS
   Stored at 4 °C

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Acknowledgments

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