ISOLATION OF MURINE SPINAL BONE MARROW

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BACKGROUND

- This protocol is used to harvest bone marrow from the spines of mice. In comparison to bone marrow isolation methods using femurs and tibias, this procedure allows for a much greater yield of cells. In addition, hematopoietic stems cells from the spine appear to have a greater proliferative and regenerative ability.

NOTES

- When sacrificing mice, avoid performing a cervical dislocation to prevent potential contamination of spinal bone marrow cells. Following sac, keep spines in chilled PBS in 50mL tubes. If not using immediately, keep at 4 C until use.
- Tools should be sterilized with 70% EtOH and cleaned vigorously of all tissue and blood after use/prior to cleaning another spine.

EQUIPMENT

- 15 cm Plates & 50mL conical tubes
- UV-treated mortar &pestle
- Alcohol Swabs (to wipe down above, allow to dry)
- 15 cm Plates & 50mL conical tubes
- Pipette gun & 10mL pipettes
- Pipettor & P1000 pipettes
- Sterilized tools (scissors & tweezers) **Note:** sterilize in 70% ethanol and then wash in sterile PBS to remove ethanol safely
- Chilled PBS
- R10 media (if going to plate BM-derived macrophages)
- 40uM cell strainer (for 50mL conical tubes)
- FBS (if banking bone marrow)
- DMSO (if banking bone marrow)

PROTOCOL

1. Sterilize biosafety cabinet and UV treat the mortar and pestle for 30 minutes.
2. Transfer spine from Falcon tube to a Petri dish containing chilled PBS.

3. Clean tissues off spine using sterilized scissors. Be very careful not to cut bone as this can compromise the bone marrow. Note: When using spines from old mice, be more careful as bones are fragile and more likely to break.
4. When spine is as clean as you can get it, cut off edges (approx. 0.25 cm) near brainstem and tail.
5. Cut spine using bone scissors into approximately 3 equal pieces.
6. Hold a piece of spine with tweezers, cut through the spine, holding the scissors vertically (as if cutting open a lobster tail) to avoid hitting the bone marrow. Once open, move scissors into a horizontal position and open in order to spread apart the sides of the spine and expose the spinal cord. Remove the spinal cord (it looks white and gooey).
7. When spinal cords from all 3 pieces are removed, place rest of spine into mortar & add approximately 5-10mL of cold PBS. Use pestle to crush pieces until PBS turns pink as this indicates bone marrow is being expelled.
8. Pipette fluid from mortar to 40uM cell strainer (placed in a 50mL conical tube) using an electronic pipettor with a 10mL pipette.
9. Repeat steps 7-8 until PBS remains clear following crushing as this indicates that all the marrow has been expelled.
10. Spin down cell suspension for 5min at 1500rpm and 4 degrees.
11. Discard the supernatant and resuspend cell pellet from each tube with 1.5 mL of pure FBS if banking bone marrow. Use a P1000 pipettor to resuspend as it is the most effective to break the cell pellet.
12. Add 0.5mL of cell suspension to each cryovial for a total of 3.
13. Add 0.5mL of 20% DMSO FBS in a dropwise fashion to each cryovial so that bone marrow cells are cryo-stored in 10%DMSO. Samples are placed on ice before transfer to Mr.Freezy.
14. The vials were placed into a freezing container (Mr.Freezy) containing isopropanol at -80°C for 24 hours.
15. The frozen vials were then transferred to the liquid nitrogen tank at -140°C for long-term storage.

Defrosting bone marrow cells

1. Warm R10 (with 15% LCM) media to 37° C and add 10mL of to a 15mL conical tube.
2. Place cryovial in water bath (37° C) and quickly thaw the cells (approx. 1-2 min).
3. Add thawed cell suspension into conical tube with warmed R10 (with 15% LCM) media.
4. Spin down cell suspension for 5min at 1500rpm.
5. Discard supernatant and resuspend cell pallet with 25mL of warmed R10 (with 15% LCM) media and plate into a 15cm dish for differentiating macrophages (Please refer to our CULTURING, FREEZING, & DEFROSTING BONE MARROW-DERIVED MACROPHAGES protocol for differentiating BMDMs).

R10 Media recipe

1. Remove 60 mL of pure RPMI media.
2. Add FBS 50 mL.
3. Penicillin/Streptomycin 1 vial (5 mL) - defrosted at 37°C, containing 10 000 U Penicillin/ml; 10 mg/ml Streptomycin
4. 200 mM L-Glutamine 1 vial (5 mL)
5. Add ~75 mL LCM if RPMI with 15% LCM is needed. [Refer to LCM protocol to know how to generate LCM].