ISOLATION OF MURINE SPINAL BONE MARROW

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BACKGROUND

- This protocol is used to harvest bone marrow from the spine 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.

EQUIPMENT

- UV-treated mortar & pestle
- Alcohol Swabs (to wipe down above, allow to dry)
- 15 cm Plates & 50mL conical tubes
- Pipette gun & 10mL pipettes
- Sterilized tools (scissors & tweezers)
  Note: may sterilize in 70% ethanol, but make sure ethanol dries before cutting into bones.
- Chilled PBS
- R10 media (if going to plate BM-derived macrophages)
- 40uM cell strainer (for 50mL conical tubes)

PROTOCOL

1. Clean tissues off spine using appropriate scissors.

Note: When using spines from old mice, be more careful as bones are fragile and more likely to break

2. When spine is as clean as you can get it, cut off edges near brainstem and tail.
3. Cut spine using bone scissors into approximately 3 equal pieces.
4. Hold a piece of spine with tweezers, cut through the spine, holding the scissors vertically (as if cutting open a lobster tail). 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.
5. When spinal cords from all 3 pieces are removed, place rest of spine into mortar & add approximately 10mL of cold PBS. Use pestle to crush pieces until PBS turns pink.
6. Pipette fluid from mortar to 40uM cell strainer (placed in a 50mL conical tube)
7. Repeat steps 5-6 until PBS remains clear following crushing.
8. Spin down cell suspension for 5min at 1500rpm.
9. Resuspend in appropriate medium.