BioGel Elicitation of Peritoneal Macrophages/Neutrophils

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Background

- This protocol describes how best to harvest a large amount of peritoneal macrophages or neutrophils from a mouse. Using this protocol, one can expect to harvest up to $5x10^7$ cells, though numbers can vary greatly

Safety Notes

- See the MSDS documentation of all materials before beginning
- It is important to maintain sterility whenever cells will be exposed to their environment.
- All work should be performed in a biosafety cabinet that has been properly sterilized by cleaning thoroughly with 70% EtOH.
- PPE required: gloves, lab coat, biosafety cabinet.
- Animal work should be performed in accordance with your institute's animal research ethics board/committee

Equipment and materials

- Equipment
    - 50mL Falcon tube
    - 150mL glass bottle
    - Centrifuge (for 50mL Falcon tubes)
    - 1mL syringe
    - 26G needle
    - 10mL syringe
    - Scissors and Forceps
    - 25G and 23G needles
    - 15mL Falcon tube
    - Cell culture dish or plate appropriate for the final application of the cells
- Materials
    - BioGel Polyacrylamide beads (we buy from Bio-Rad)
    - Sterile PBS
    - Ice-cold sterile PBS (for peritoneal lavage)
    - RPMI + 10% FBS + L-glut + Pen/Strep

Protocol

A. Preparing BioGel and Injecting Mice

- Wash 2g of BioGel beads in 50mL of sterile, endotoxin-free PBS or water.
- Pellet by centrifugation for 5min at 400g and resuspend in 100mL of PBS to get a 2%(w/v) solution.
    - Autoclave before use.
- Inject mice intraperitoneally with 1mL of the BioGel solution using a 1mL syringe and 26G needle (or to your preference)
- Wait 12-24 hours (for neutrophils) or 4-5 days (for macrophages)

B. Peritoneal Lavage (for video: http://www.jove.com/video/1488/isolation-of-mouse-peritoneal-cavity-cells)

- Sacrifice mice by cervical dislocation following gas anaesthesia or CO_2 inhalation
- Cut a small slit at the lowest aspect of the abdomen, avoiding cutting through the peritoneum
- Pull the skin to expose the peritoneum
- Inject 10mL of ice-cold PBS into the peritoneal cavity using a 10mL syringe and 25G needle
- Massage the peritoneum in order to encourage cells to detach from the peritoneal wall
- Using a 10mL syringe with a 23G needle, collect as much fluid as possible.
    - If necessary, you can cut the peritoneum and use a plastic pasteur pipet to finish collecting the fluid
- Deposit the fluid containing the cells in a 15mL Falcon tube

C. Culturing Cells

- Wash the cells 2x in complete RPMI media or PBS, resuspending in complete RPMI after the last wash
- Wash steps should be performed using 10mL of fluid and centrifugation at 1500rpm for 5min.
    - Cells can be passed through a 70µm cell strainer in order to remove beads. **This step is not necessary for the culture of macrophages but is for neutrophils.**
- Count cells using hemacytometer
- For macrophages, seed cells on a bacteriological plate in plenty of warm complete RPMI media. Allow to sit for 2h at 37˚C with 5% CO_2 and then wash with warm media to remove any beads or unwanted cells. After this, ~98-100% of your cells should be mature macrophages.
- For neutrophils, seed directly into cell culture plates for use. Purity should be fine, however flow sorting or magnetic bead separation may be necessary to obtain a completely pure culture.

CLEAN-UP INSTRUCTIONS

- All leftover materials and used media should be disposed of in 10% bleach for 30min before flushing down sink with plenty of running water
- Solid materials, such as Falcon tubes and Cryovials, should be placed in Biohazard bag and disposed of appropriately
- If a vacuum pump was used, make sure to flush the tubes with 10% bleach for 1min
- Mice should be disposed of as per your animal use protocols