Spence Laboratory - University of Michigan

7/2/2018

Human Tissue Digestion (for downstream FACS isolation of ECs)

Digestion of tissue to single cell state

1. Mince tissue to <1 mm^3. Incubate in 50 mL conical with 9 mL collagenase II and 1 mL dispase (per gram of tissue). See below for reagent prep.
    - Incubate at 37°C for 30 minutes - **continuous** agitation
2. Add 75 µL DNase (per 10 mL of digestion solution)
    - Incubate at 37°C for 30 minutes - **continuous** agitation
3. Add 5 mL EC isolation medium (per 10 mL of digestion solution). Pass cells through a 100 µm cell strainer to remove undigested tissue. Optional rinse with an additional 5 mL EC isolation medium. Collect in a clear 50 mL conical.
    - Centrifuge at 400g for 5 minutes (RT)

Removal of RBCs and dead cells

4. Resuspend cells in 10 mL Ficoll cell separation medium (per gram starting material).
5. Layer suspension on 7.5 mL Ficoll-Paque PLUS (prewarmed to RT). To layer, carefully tilt conical on its side and *slowly* pipette the cell suspension down the side of the tube. Centrifuge at 400g for 20 minutes at 18°C **with as slow of acceleration as possible and without brakes**…this will end up taking approximately 45 minutes in total.
    - Do not disturb the Ficoll-Paque - will ruin density gradient
    - Put cells on **ice** immediately after centrifugation
6. Transfer interphase to a fresh tube and add 2 volumes of EC isolation medium. Remove top layer to more easily access the middle layer containing cells of interest.
    - Centrifuge at 400g for 5 minutes
7. Resuspend the cells in an appropriate medium depending on downstream application.

<table>
<tr><th>EC Medium</th><th>EC Isolation Medium</th></tr>
<tr><td>

- 78 mL RPMI-1640
- 20 mL FBS
- 1 mL penicillin-streptomycin (10,000 U/mL; 10,000 mg/mL)
- 1 mL L-Gln (200mM - not included if RPMI contains glutamine already)

*Filter through a 0.2 µM filter
*Store at 4°C

</td><td rowspan="2">

Component A (80% vol/vol)

- 74 mL RPMI-1640
- 1 mL L-Gln (200 mM - not included if RPMI contains glutamine already)
- 1 mL penicillin-streptomycin (10,000 U/mL; 10,000 mg/mL)
- 1 mL sodium pyruvate (100 mM)
- 1 mL nonessential amino acids (10 mM)
- 1 mL HEPES (1 M)
- 1 mL MEM vitamins (100x)
- 20 mL FBS

Component B (20% vol/vol)

- **EC Medium**

*Store at 4°C for 1 week

</td></tr>
<tr><td>

Ficoll-Paque Separation Medium

- **EC Medium**
  - 1% (vol/vol) 2 M sodium citrate

</td></tr>
</table>

- Collagenase Type II Solution: 0.1% (w/v) in PBS, Filter sterilized. Made fresh each time.
- Dispase Solution: Dissolve dispase to 2.5 units/mL in PBS. Filter sterilized. Made fresh each time.

Protocol adapted from van Beijnum, J. R., Rousch, M., Castermans, K., van der Linden, E., & Griffioen, A. W. (2008). Isolation of endothelial cells from fresh tissues. *Nature Protocols*, *3*(6), 1085–1091. http://doi.org/10.1038/nprot.2008.71