Systems *Bioengineering* Laboratory

Subculture of Conditionally Immortalized Human Podocytes

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Materials

- Glacial (17.4N) acetic acid (Sigma, Cat: A6283)
- Type I (rat tail) collagen (BD, Cat: 354236)
- RPMI-1640 Medium (Gibco, Cat: 11875-119)
- ITS-G, insulin-transferrin-selenium (Gibco, Cat: 41400-045)
- Fetal calf serum (Gibco, Cat: A3160602)
- Pen-Strep (Gibco, Cat: 1514-122)
- PBS (Gibco, Cat: 14190-250)
- 0.05% Trypsin-EDTA (Gibco, Cat: 25300-054)

Methods

1. All procedures must be carried out aseptically inside a biological safety laminar flow cabinet (tissue culture hood).

Thawing Cells

2. Before thawing or passaging cells, surface of the culture vessel(s) must be coated with sterile 0.1 mg/mL type I collagen and culture media must be prepared. Depending on the step below, preparations may take 1-3 hours.

   2.1. Prepare sterile 0.02N acetic acid solution: add 869 ml water and 1 ml glacial acetic acid (17.4N) in autoclavable bottle and autoclave under liquid settings (45-75 minutes, depending on the autoclave model) making sure that the cap of the bottle is loose and the cap is labeled with autoclave tape prior to start. After completion, promptly tighten the cap and wait for the solution to completely cool down before using.

   2.2. Dilute main collagen stock (dilution ratio depends on the concentration of the stock, which varies from batch to batch) such that final concentration is 0.1 mg/mL.

   For example: Collagen stock with concentration of 4.3 mg/mL needs to be diluted 43X, so add 2.325 mL of collagen into 97.675 mL of 0.02N acetic acid solution.

   2.3. Coat the surface(s) with necessary volume of collagen solution (e.g., 4 mL for 150 cm^2 flask; 2.5 mL for 75 cm^2 flask; 0.75 mL for 10 cm dish) for 60 minutes inside a 37ºC cell culture incubator.

   2.4. Wash the surface(s) with room temperature PBS, twice. Aspirate PBS.

   Note: Culture surfaces can be kept at this state for about two weeks. We clearly record the coat date and use these vessels within a week.

   2.5. Add 50 mL of fetal calf serum, 5 mL of Pen-Strep, and 5 mL ITS-G into a 500 mL bottle of RPMI-1640 cell culture media. This “10% FBS Media” will be used to culture the conditionally immortalized cells under all conditions.

3. Pre-warm media by pipetting adequate amount into the culture vessel (e.g., 25 mL for 150 cm^2 flask; 15 mL for 75 cm^2 flask; 7 mL for 10 cm dish) and placing inside a 33ºC cell culture incubator for 15 minutes.

   Note: We freeze 1 million cells per tube and thaw these into a single 150 cm^2 flask or two 75 cm^2 flasks.

4. Thaw cells by rapidly placing them inside a 37ºC water bath (make sure not to completely submerge the tubes to prevent potential contamination) for 30-60 seconds checking for thaw status every 10 seconds.

5. Pipette freshly thawed cells into the pre-warmed media, evenly distributing over the surface. Place the vessel into the 33ºC cell culture incubator under 5% CO_2.
6. Change media every 2-3 days. Always pre-warm media to 37ºC.

Passaging Cells

7. Cells must be passaged before reaching 85-90% confluence. To passage cells onto a new culture vessel, coat the new vessels according to Step 1 above, and pre-warm culture media and PBS to 37ºC and trypsin to room temperature. Do not heat trypsin as this will cause it lose its efficacy.
8. Aspirate media and wash cells with warm PBS
9. Aspirate PBS; add trypsin-EDTA (e.g., 4.5 mL for 150 cm^2 flask; 3 mL for 75 cm^2 flask; 1.5 mL for 10 cm dish) and place into 37ºC incubator for 5 minutes
10. After 5 minutes, tap to the side of the vessel to help dislodge cells (be careful not to splash the solution around) and check on a microscope that all cells have been dislodged
11. Collect trypsinized cells into a 50 mL conical tube and wash the vessel with more media three times, collecting all media in the same vessel (final volume should be roughly 10X of the original trypsin amount). If necessary, cells should be counted at this point.
12. Spin down cells at 250g for 5 minutes at 4ºC.
13. Aspirate supernatant, resuspend cells with media, plate onto new vessels, and place back into 33ºC incubator

    Note: We normally passage cells 1:3 to 1:6 every third day.

Plating Cells for Differentiation

14. Repeat steps 7-11 and measure cell density using an automated cell counter and/or hemocytometer, and calculate total number of cells
15. Spin down cells at 250g for 5 minutes at 4ºC and resuspend with the appropriate volume commensurate with the appropriate plating surface area

    15.1. For regular tissue culture surfaces, we plate podocytes at a concentration of 2,500 – 5,000 cells/cm^2. We use the higher end of this spectrum for experiments with cell-free assays, such as RNA or protein collection. We use the lower end of this spectrum for imaging experiments where accurate segmentation of individual cell spreading area is critical.
16. Plate cell onto new vessels and place into 37ºC incubator
17. Culture cells for 7-10 days changing mediate every other day.